Investigating the role of excitatory circuitry in the orbitofrontal cortex in social cognition

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INVESTIGATING THE ROLE OF EXCITATORY CIRCUITRY IN THE ORBITOFRONTAL CORTEX IN SOCIAL COGNITION

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Abstract

Impaired social cognition is a common component of many neurodevelopmental conditions. These impairments impair quality of life. However, the factors shaping social development and accompanying neural circuitry are not well understood.

The brain regions that contribute to social cognition are called the "social brain". The orbitofrontal cortex is one of the brain regions that constitute the "social brain". Damage to the orbitofrontal cortex causes profound deficits in social cognition, including disinhibition and aggression. Dysfunctional circuitry in the orbitofrontal cortex may contribute to impaired social cognition in neurodevelopmental disorders. However, the role played by neuronal circuitry in the orbitofrontal cortex to social cognition is unclear.

I sought to examine the role of excitatory circuitry within the orbitofrontal cortex in social cognition. I first explored the contribution of excitatory circuitry in the orbitofrontal cortex to impaired social behaviour in *Nrxn1α* KO mice. These mice carry a genetic microdeletion that is a risk factor for multiple neurodevelopmental disorders, including autism and schizophrenia. Previous experiments have demonstrated that these mice display impaired social behaviour. I found that impaired social behaviour in *Nrxn1α* KO mice was accompanied by a reduction in short-term facilitation of pyramidal synapses.

The second aim of my thesis was to investigate the role of sensory experience in shaping the development of social cognition and accompanying "social brain" circuitry. This was achieved by depriving rodents of whisker input during a sensitive period of social development. I found that a short period of whisker deprivation led to long-lasting changes in rodent play behaviour. However, this was not accompanied by changes in excitatory circuitry in the orbitofrontal cortex.

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Statement of contribution

All of the work described in this thesis is my own unless otherwise stated. Alastair Kirby assisted in whisker trimming rats. Data acquisition and analysis programmes for electrophysiological recording data were written by Dr Gerald Finnerty.

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1 Introduction

1.1 An introduction to social cognition

1.1.1 What is social cognition?

Social interaction is a vital aspect of our everyday lives. The ability to interact successfully with others is crucial to our mental and physical wellbeing. Positive social interaction is rewarding and increases wellbeing (Pinquart & Sörensen, 2000). In contrast, the absence of positive social interaction has a highly detrimental effect on mental and physical health (Eisenberger & Cole, 2012; Holt-Lunstad, Smith, & Layton, 2010).

As a result, we possess cognitive processes dedicated to the encoding, storage and decoding of social information. These processes are called "social cognition". These cognitive processes enable us to interpret the intentions, actions, and feelings of others, understand our own feelings, and select appropriate responses during social interaction.

Many different psychological processes have been recognised as contributing to social cognition. However, a definitive classification of the structure and domains of social cognition has not been achieved (Happé, Cook, & Bird, 2017). There is little consensus with regards to what constitute the core domains of social cognition. The National Institute of Mental Health's research domain criteria identify four core domains of social cognition which include: affiliation/attachment, social communication, perception and understanding of the self, and perception and understanding of others. Beer & Ochsner (2006) divide social cognition into knowledge of the actions and intentions of others, knowledge of our own emotional states, and knowledge of the declarative or procedural social "rules" concepts that guide our responses during social interactions (Beer & Ochsner, 2006). In contrast, Henry et al (2015) separates social cognition into the four domains of affective empathy, interpreting

the intentions of others, social perception, and performing "appropriate" social behaviour. Other classifications of social cognition have also been proposed (Green et al., 2008; Happé & Frith, 2014; Pinkham et al., 2014).

The lack of agreement over the core cognitive processes underlying social cognition reflects both an absence of understanding of the inter-relatedness and inter-dependence of different social cognitive domains, as well as lack of consensus over use of terminology and definitions (Happé et al., 2017). However, whilst it is still unclear to what extent different socio-cognitive rely on overlapping or distinct mechanisms, a number of processes have been identified as contributing to social cognition. These are described below.

1.1.1.1 Social attention

Social attention refers to the attention paid to social stimuli through endogenous or exogenous attention. Multiple studies indicate that social stimuli such as faces preferentially capture attention, and are processed more rapidly, relative to non-social stimuli or scrambled social stimuli (Johnson, Dziurawiec, Ellis, & Morton, 1991a; Purcell & Stewart, 1988; Shah, Happé, Sowden, Cook, & Bird, 2015). A second facet of social attention is "joint attention". This refers to the shared focus of attention with another individual onto another stimulus by means of social gestures, such as pointing, or eye gaze (Scaife & Bruner, 1975; Mundy & Newell, 2007).

1.1.1.2 Social motivation and reward

Social motivation is defined as the set of mechanisms that cause us seek out, and interact with others, to find interaction rewarding, and to maintain social bonds (Chevallier, Kohls, Troiani, Brodkin, et al., 2012). Social interaction with conspecifics is usually rewarding; individuals will exert effort to acquire social rewards (Hayden, Parikh, Deaner, & Platt,

2007). The rewarding nature of social interaction is thought to promote affiliation and attachment to conspecifics.

1.1.1.3 Recognition of conspecifics

Recognition of other social agents relies of perceptual processing of the sensory features of social stimuli. In humans, recognition of faces is a key component of recognition of conspecifics (Little, Jones, & DeBruine, 2011). However, other sensory features, such as vocal tone and prosody, also contribute to recognition of conspecifics (Lass, Hughes, Bowyer, Waters, & Bourne, 1976; Papcun, Kreiman, & Davis, 1989).

1.1.1.4 Emotion recognition

Social interaction requires the ability to recognise and make inferences about other people's feelings and emotional states. This is achieved by recognising and interpreting sensory cues that carry social meaning. For example, facial expressions are an important source of information about the emotional states of others. Six basic emotions (happiness, disgust, anger, fear, sadness and surprise) have been recognised on the basis of facial expressions (Ekman & Oster, 1979; Ekman, 1992), although we are also capable of detecting more subtle emotional states, such as desire, or determination from facial expressions (Baron-Cohen, Wheelwright, Hill, Raste, & Plumb, 2001). In addition, other sensory cues also guide perception of emotional states. This includes cues such as body posture and motion, auditory tone, and verbal communication (Clarke, Bradshaw, Field, Hampson, & Rose, 2005; Coulson, 2004; Scherer, Banse, Wallbott, & Goldbeck, 1991).

1.1.1.5 Empathy

Empathy is the ability to recognise and share the emotional state of another social agent. This has been further divided into "cognitive" empathy and "affective" empathy (Shamay-Tsoory,

Aharon-Peretz, & Perry, 2009). “Cognitive” empathy is defined as the ability to understand and take the perspective of another social agent. In contrast, “affective” empathy refers to the ability to feel the same emotions as a social agent, which is based on emotional contagion.

1.1.1.6 Action understanding and imitation of social agents

These are the processes that enable us to recognise the action performed by a social agent, and to reproduce this action ourselves. Imitation is an important component of social interaction. During social interactions, we tend to non-consciously reproduce the gestures, mannerisms, and behaviours of others (Chartrand & Bargh, 1999). This is called “mirroring”. Imitation is thought to be an important for promoting affiliation; we tend to like those who behave similarly to us, and we tend to unconsciously imitate social agents when we like them more (Chartrand & Bargh, 1999). Perception and recognition of the actions performed by social agents is a precursor to reproducing the same behaviours.

1.1.1.7 Attributing mental states

“Mentalising” or “Theory of Mind” is the ability to attribute mental states to others and to infer the emotions, thoughts and intentions of others (Wimmer, 1983). This requires the ability to differentiate between one’s own thoughts, beliefs and feelings, and those of other social agents (the self/other distinction).

1.1.1.8 Knowledge of social rules and concepts

Successful social interaction also relies on the learning, storage and retrieval of social rules, normal and concepts that guide social situations. This requires recognition of social contexts, flexible retrieval and application of social rules that are appropriate to the context, and knowledge of the consequences of social behaviour. These rules and norms are often

specified by culture and must be learnt through social experience (Moll, Zahn, de Oliveira-Souza, Krueger, & Grafman, 2005).

1.1.1.9 Self-regulation of emotions and social behaviour

This is the ability to select, initiate and control social behaviours according to the social environment (Bachevalier & Loveland, 2006). Recognition and interpretation of one's own emotional states is likely to be required as a precursor to controlling emotional states. In addition, "social referencing", the tendency to use the reactions of other social agents as an indicator of how to behave, is also a component of the self-regulation of social behaviour (Walden & Ogan, 1988).

1.1.2 The relationship between social and non-social cognition

The structure of the cognitive processes involved in social cognition remains largely undefined. It is still unclear what constitute the "core" latent cognitive processes underlying different aspects of social behaviour. In addition, it is unclear how different facets of social cognition relate to one another, or to other, non-social cognitive processes (Happé et al., 2017).

One area that has been particularly debated is the question of whether social cognition relies on domain-specific, modular processes, or domain-general processes that are also invoked during non-social cognition. A number of researchers have argued that evolutionary pressures have led to the development of domain-specific processes for social cognition (Adolphs, 2006; Brothers, 1996). According to this theory, living in social groups has promoted the evolution of domain-specific social learning mechanisms. The idea that social stimuli are "special" and undergo specialised processing is supported by findings that we preferentially attend to and process social stimuli relative to non-social stimuli (Fletcher-Watson, Findlay, Leekam, & Benson, 2008; Purcell & Stewart, 1988; Shah et al., 2015; Tomalski, Csibra, &

Johnson, 2009). Other evidence for a separation of social and non-social cognitive processes can be found in functional magnetic resonance imaging (fMRI) studies investigating hierarchical learning. Such studies have identified brain regions in which variations in blood-oxygen-level dependent (BOLD) activity and grey matter specifically predict learning of social, as opposed to non-social, hierarchies (Kumaran, Banino, Blundell, Hassabis, & Dayan, 2016; Kumaran, Melo, & Duzel, 2012).

Others have disputed this claim. For example, Heyes (2012a) argued that all learning, including social learning, relies on the same basic associative learning mechanisms. The notion that the same processes underlie social and non-social learning is supported by correlations between the rate of learning in social and non-social tasks across species (Boogert, Giraldeau, & Lefebvre, 2008; Reader & Laland, 2002). The proposal that there is an innate "social" module responsible for our ability to imitate conspecifics has also been refuted by evidence demonstrating that domain general associative learning mechanisms are responsible for imitation (Anisfeld, 1979; Cook, Press, Dickinson, & Heyes, 2010; Jones, 2009; Ray & Heyes, 2011).

The notion that there are domain-specific social cognitive processes receives some support from functional magnetic resonance (fMRI) studies. Certain brain regions appear to be preferentially involved in processing social stimuli. For example, a region of the fusiform gyrus (the "fusiform face area") is preferentially activated during the viewing of faces relative to scrambled or inverted faces, or other face-like stimuli (Kanwisher, McDermott, & Chun, 1997). A portion within the superior temporal sulcus appears specialised for the viewing of biological motion (Saygin, 2007). Moreover, distinct neural structures were associated with learning in a social learning task compared to a non-social reward learning task (Behrens, Hunt, Woolrich, & Rushworth, 2008).

However, the extent to which these neural structures are truly specialised for performing social computations is contended. Studies indicate that the fusiform face area can become activated by stimuli with which we have "expertise", or by made-up stimuli after training (Gauthier, Skudlarski, Gore, & Anderson, 2000; Gauthier, Tarr, Anderson, Skudlarski, & Gore, 1999). In addition, it has been argued that similar computational mechanisms may be involved in social and non-social cognition despite the use of distinct brain structures (Behrens et al., 2008). Beer & Ochsner (2006) argued that the neural processes involved in social cognition are likely to be, for the most part, domain-general, on the basis that the brain structures and neural computations required for social cognition do not appear specific to social cognition.

In sum, the relationship between social and non-social cognition remains unclear. Some evidence at the neuropsychological level supports the notion of a dissociation between social and non-social cognitive processes (Pellicano, 2007). However, double dissociations are scarce, and when reported, are complicated by the fact that there may be sub-threshold impairments. Moreover, when they are observed in developing children, it is unclear whether "spared" domains are truly spared, or whether compensatory mechanisms have arisen as a result of atypical development (Karmiloff-Smith, 2009; Karmiloff-Smith, Scerif, & Ansari, 2003a).

1.1.3 Social cognition in psychiatric and neurological disorders

Impairments in social cognition are observed across many neurological and psychiatric conditions (Henry, von Hippel, Molenberghs, Lee, & Sachdev, 2015). Altered social behaviour is a common feature in neurological disorders, such as traumatic brain injury, brain tumours, stroke, and neurodegenerative disorders. In these cases, abnormal social behaviour frequently manifests as heightened aggression and emotional reactivity, lack of understanding of social situations and "inappropriate" behaviour, and reduced empathy.

Impaired social cognition is particularly prevalent in conditions that arise during development. This includes neurodevelopmental disorders with a genetic component, as well as childhood traumatic brain injury (Anderson, Damasio, Tranel, & Damasio, 2000; Korkmaz, 2011; Mateer & Williams, 1991). The prevalence of social impairments in neurodevelopmental disorders suggests that social cognition is highly vulnerable to disruption during development, and highlights the need to understand how the brain supports social cognitive development.

Whilst altered social behaviour is observed across numerous developmental disorders, this is characterised by a great degree of heterogeneity of symptoms. Indeed, the facets of social cognition that are affected can vary both within and across conditions. A description of neurodevelopmental disorders that commonly carry a socio-cognitive impairment is presented below.

1.1.3.1 Autism spectrum disorders

Autism spectrum disorder consists of a range of neurodevelopmental disorders characterised by the presence of altered social behaviours, impaired language and communication, and restricted interests with repetitive, stereotyped behaviours (Diagnostic and Statistical Manual of Mental Disorders (DSM-V), American Psychiatric Association, 2013). In addition, the diagnosis is often comorbid with other conditions such as epilepsy, intellectual disability, anxiety, tics, sleep disorders, attention deficit hyperactivity disorder, and gastrointestinal problems. The label of autism spectrum disorders as defined by the most recent edition of the DSM encompasses the previous diagnoses of autism, Asperger syndrome, pervasive developmental disorder not otherwise specified (PDD-NOS) and childhood disintegrative disorder (American Psychiatric Association, 2013). Autism spectrum disorders affect approximately 1% of children in the UK, and 1 in 68 children in the US (Baird et al., 2006).

Autism spectrum disorders possess a strong genetic component, with heritability estimates ranging between 38 % and 90 % (Bailey et al., 1995; Folstein & Rosen-Sheidley, 2001; Hallmayer et al., 2011; Ronald & Hoekstra, 2011; Sandin et al., 2014). The genetic aetiology of autism spectrum disorders is complex, with both common, small-effect variants and rarer, larger-effect copy number variants contributing to aetiology (Geschwind & Flint, 2015; Manolio et al., 2009a). Syndromic autism spectrum disorders are conditions caused by a known genetic abnormality, such as Fragile X syndrome, tuberous sclerosis, Prader-Willi syndrome, Angelman syndrome and Rett syndrome (Sztainberg & Zoghbi, 2016). These make up approximately 5% of autism spectrum disorder cases (Betancur, 2011).

The symptoms of autism spectrum disorders are heterogeneous and vary across individuals. Whilst abnormal social behaviour is a core diagnostic feature of autism spectrum disorders, this can manifest in different ways. Whilst certain individuals display avoidance or lack of interest in engaging in social interaction, other individuals demonstrate a heightened and persistent interest in social interaction coupled with "odd" behaviours (Wing & Gould, 1979). Studies that have further investigated the nature of altered social behaviour in autism spectrum disorders have revealed a range of impairments across multiple facets of socio-cognitive behaviour, including deficits in making and maintaining eye contact, joint attention, social motivation, pretend play, moral and social judgements, imitation, emotion recognition, and in inferring other people's mental states (Ashwin, Chapman, Colle, & Baron-Cohen, 2006; Baron-Cohen, Jolliffe, Mortimore, & Robertson, 1997; Baron-Cohen, Leslie, & Frith, 1985; Charman et al., 1997; Golan, Baron-Cohen, Hill, & Rutherford, 2007; Happé, 1994; Jarrold, 2003; Moran et al., 2011). In addition, there is a high degree of variability in social symptoms across individuals.

One of the most consistently observed impairments in individuals with autism spectrum disorders is impaired theory of mind (Baron-Cohen et al., 1985). Children with autism spectrum disorders typically fail tests of theory of mind. Moreover, children who are able to pass theory of mind tasks pass at a later age than typically developing children. High functioning autistic individuals are able to pass simpler tests, but often display deficits with more complicated second-order or third-order theory of mind tests (Baron-Cohen, O'Riordan, Stone, Jones, & Plaisted, 1999; Happé, 1994). In addition, even in high-functioning adults with Asperger's syndrome who are capable of passing more complex theory of mind tasks, changes in strategy are observed (Senju, Southgate, White, & Frith, 2009).

Numerous theories have been proposed to account for social symptoms in autism spectrum disorders. One theory suggests that autism spectrum disorders are characterised by a deficit in social motivation, on the basis that autistic individuals display reduced attention to social stimuli and reduced motivation for social interaction (Chevallier et al., 2012). However, this is not seen in all individuals with autism spectrum disorders. Indeed, some individuals display persistent attempts to engage in social interaction, but fail to interact successfully with others (Wing & Gould, 1979). A separate theory proposes that social symptoms in autism spectrum disorders result from a deficit in a shared mechanism responsible for imitation, empathy, and theory of mind (Rogers & Pennington, 1991). However, multiple studies have called into question the notion that individuals with autism possess impaired empathy or imitative skills (Bird, Leighton, Press, & Heyes, 2007; Cook & Bird, 2012; Sowden, Koehne, Catmur, Dziobek, & Bird, 2016).

1.1.3.2 William's syndrome

William's syndrome is a neurodevelopmental syndrome caused by a large deletion in the long arm of chromosome 7. Individuals with William's syndrome display symptoms across

physical and mental domains. This includes symptoms such as unusual facial features, heart defects, gastrointestinal problems, overactive muscle reflexes, anxiety, language delays, intellectual disability, and social symptoms (Martens, Wilson, & Reutens, 2008).

Individuals with William's syndrome display an unusual social phenotype. Initially, social cognition was thought to be intact in children with William's syndrome, despite pronounced intellectual impairments. Indeed, unlike children with autism spectrum disorders, children with William's syndrome show great interest in social stimuli (Riby & Hancock, 2009). However, more recent studies have demonstrated that their social behaviours are also altered. Children with William's syndrome display overly disinhibited social behaviour, with an exaggerated tendency to approach others, and without awareness of potential danger (Doyle, Bellugi, Korenberg, & Graham, 2004; Jones et al., 2000). This may be driven by reduced ability to recognise aversive social cues. Individuals with William's syndrome display selective deficits in the ability to recognise negative facial expressions such as anger or sadness (Gagliardi et al., 2003; Plesa-Skwerer, Faja, Schofield, Verbalis, & Tager-Flusberg, 2006). In addition, some individuals with William's syndrome display impulsivity, aggressive behaviours, and disobedience (Einfeld, Tonge, & Florio, 1997; Einfeld, Tonge, & Rees, 2001; Udwin & Yule, 1991).

1.1.3.3 Schizophrenia

Schizophrenia is a neurodevelopmental condition that is characterised by the presence of positive symptoms (hallucinations, delusions), negative symptoms (apathy, anhedonia, and social withdrawal (DSM-V, American Psychiatric Association, 2013). Schizophrenia affects 0.7% of the population worldwide and usually emerges in late adolescence (McGrath, Saha, Chant, & Welham, 2008). Heritability studies indicate that schizophrenia is highly heritable,

with heritability estimates of around 80% (Sullivan, Kendler, & Neale, 2003). Like autism spectrum disorders, schizophrenia possesses a complex genetic aetiology.

In addition to positive and negative symptoms, individuals with schizophrenia also display prominent deficits in social and non-social cognition, which precede the first episode of psychosis (Couture, Penn, & Roberts, 2006; Gold & Harvey, 1993; Green, 1996; Green, Kern, & Heaton, 2004a; Mohamed, Paulsen, O'Leary, Arndt, & Andreasen, 1999; Penn, Corrigan, Bentall, Racenstein, & Newman, 1997). Deficits are particularly apparent in the abilities that enable us to infer other people's feelings and mental states. This includes deficits in the ability to correctly judge facial expressions and recognise emotions, and in theory of mind (Brune, 2005; Frith & Corcoran, 1996). In addition, individuals with schizophrenia display heightened aggression (Hoptman et al., 2010).

1.1.3.4 Turner's syndrome

Turner's syndrome is a genetic condition affecting females which is caused by the total or partial absence of the X chromosome (Ford, Jones, Polani, De Almeida, & Briggs, 1959). Individuals with Turner's syndrome display difficulties in spatial reasoning and mathematics. In addition, individuals with Turner's syndrome often display abnormal social cognition. This is characterised by difficulty with emotion recognition and gaze perception (Burnett, Reutens, & Wood, 2010; McCauley, Kay, Ito, & Treder, 1987).

1.1.3.5 Summary

Deficits in social cognition are apparent across numerous neurodevelopmental disorders, including autism spectrum disorders, schizophrenia, William's syndrome, Turner's syndrome, as well as in cases of syndromic autism spectrum disorders (e.g. Angelman syndrome, Prader-Willi syndrome, tuberous sclerosis, Fragile X syndrome and Rett's syndrome). In these conditions, impairments can occur across multiple facets of social

cognition. There are differences between the social phenotypes displayed across individuals with distinct neurodevelopmental conditions. For example, individuals with William's syndrome display hypersociability, whilst individuals with autism spectrum disorders often display reduced sociability (Chevallier et al., 2012; Jones et al., 2000) However, the social cognitive impairments seen across neurodevelopmental disorders also display many common features. For instance, deficits in the ability to recognise emotions from social cues and to understand social situations are present across many conditions (Ashwin et al., 2006; Brune, 2005; Gagliardi et al., 2003; Plesa-Skwerer et al., 2006). Deficits in theory of mind are also seen across multiple conditions, including both autism spectrum disorders and schizophrenia (Baron-Cohen et al., 1985; Frith & Corcoran, 1996a). Finally, lack of emotional regulation, disinhibition, and aggression can feature in many conditions (Einfeld et al., 1997; Hoptman et al., 2010; Udwin & Yule, 1991). This similarity of symptoms has led to a greater focus on studying symptom clusters at the level of neural circuitry, rather than studying neural circuit disruption in each condition separately (Insel & Cuthbert, 2015).

1.2 Social cognitive development

1.2.1 Social cognitive development in typically-developing children

There has been much interest in studying the trajectory of social cognitive development. This is partially driven by the fact that many neuro-genetic developmental disorders present with altered social cognition (Happé & Frith, 2014). A particular issue of interest has been to what extent social cognitive abilities are innately determined or learnt via experience, as this has different implications for our understanding of neurodevelopmental disorders. There is evidence to support a role for both innate and experience-driven mechanisms in social development. In addition, studies indicate that social cognition develops slowly. While sensory and motor abilities are mainly assimilated during early infancy, social cognition

exhibits a protracted period of development which extends throughout childhood and adolescence (Choudhury, Blakemore, & Charman, 2006). This protracted development may explain why social cognition is vulnerable to disruption during development. The trajectory of social cognitive development is described briefly below.

Evidence suggests that certain facets of social cognition develop very rapidly in early infancy, and may even be present from birth. Elements of social cognition can be observed even in neonates. For example, newborns already display a preference for looking at social stimuli, such as face-like stimuli (Johnson et al., 1991a; Valenza, Simion, Cassia, & Umiltà, 1996) and eye gaze (Batki, Baron-Cohen, Wheelwright, Connellan, & Ahluwalia, 2000). In addition, neonates attend preferentially to biological motion (Simion, Regolin, & Bulf, 2008), as well as to their mother's face (Pascalis, de Schonen, Morton, Deruelle, & Fabre-Grenet, 1995; Walton, Bower, & Bower, 1992). Finally, some evidence suggests that newborns are capable of imitating social agents (Meltzoff & Moore, 1977; Andrew N. Meltzoff & Moore, 1983). However, whether or not neonates are capable of true imitation has been highly debated, with numerous studies reporting non-replication, or finding that imitation in newborns is specific to tongue protrusion (Anisfeld, 1996; Anisfeld et al., 2001; Hayes & Watson, 1981).

Attention to social stimuli and to the structure of social interaction continues to be refined in the first year of life. Young infants display basic recognition of the reciprocity of social interaction, showing distress when this reciprocity is broken (Gusella, Muir, & Tronick, 1988). Infants also begin to show sensitivity to "social cues" that indicate intent or carry social meaning, such as eye gaze and facial expressions. For example, infants display preference for listening to someone calling their own name (Mandel, Jusczyk, & Pisoni, 1995). At 4 months, infants are able to process gaze direction, and discriminate between

open/closed eyes and direct/averted gaze (Vecera & Johnson, 1995). Finally, infants also begin to display recognition of the valence of social stimuli; electroencephalogram electrophysiological event-related potential recordings indicate that 7-9 month old infants are able to discriminate between sad versus neutral facial expressions (Leppänen, Moulson, Vogel-Farley, & Nelson, 2007).

Across the first and second year of life, infants continue to develop awareness of the reciprocity of social behaviour and of the mental states of others. 9-12 month old infants begin to use social gestures such as pointing to direct the attention of an observer and communicate intent (Tomasello, Carpenter, & Liszkowski, 2007). This is followed by development of joint attention, which is the ability to track the gaze of social agents and share the focus of their gaze (Mundy & Newell, 2007). Finally, infants begin to use social cues to guide their own behaviours, a process called "social referencing". For example, 12-month old infants avoid objects if their mothers have displayed fear towards the object (Vaish & Striano, 2004). There is also some evidence from gaze studies using habituation paradigms that infants around 1 year of age display sensitivity to the mental states of others (Baillargeon, Scott, & He, 2010). These behaviours indicate a growing appreciation of social interaction as a means to communicate intent, as well as some sensitivity other people's intentions and emotional states.

Socio-emotional and affiliative behaviours develop in parallel. Parental attachments continue to form and strengthen (Ainsworth, 1979). 9-12 month-old infants display separation anxiety, as well as affiliative 'teasing' behaviours (Reddy & Mireault, 2015). The ability to recognise emotion also become more refined; one study indicates that 16-19-month-old infants are able to detect inauthentic emotions (Walle & Campos, 2014).

With the transition into later infancy and early childhood, more complex aspects of social cognition emerge. The ability to attribute mental states to others is a key feature of social cognition and is called Theory of Mind or "mentalising" in the psychological and psychiatric literature (Frith & Frith, 2012). By late infancy, children demonstrate an increased ability to understand and predict the mental states of others. For example, 2 year old children engage in pretend play (Leslie, 1987), spontaneous helping and cooperative behaviours (Buttelmann, Carpenter, & Tomasello, 2009; Warneken, Gräfenhain, & Tomasello, 2012; Warneken & Tomasello, 2007). An understanding of theory of mind is also observed from around 2 years of age. The exact age at which children first display theory of mind has been hotly debated (Baillargeon et al., 2010), but it is clearly present by children aged 3-5. Around 3-5 years, children also display an understanding of the concept of fairness and morality (Vaish, Carpenter, & Tomasello, 2010). In addition, young children display increased emotional processing. The period around 11 months to 2 years, is characterised by the emergence of social emotions such as jealousy (Masciuch & Kienapple, 1993). This is followed by the development of social concepts, such as morality and fairness (Kochanska, Gross, Lin, & Nichols, 2002; Vaish et al., 2010). Middle childhood is characterised by further development of emotional processing, morality judgements, understanding of "social emotions" and attribution of mental states (e.g. second-order false belief tasks) (Cushman, Sheketoff, Wharton, & Carey, 2013; Eisenberg, 2000).

Social cognition continues to undergo extensive changes during adolescence. Adolescence is characterised by greater self-awareness, increased perspective-taking abilities (Choudhury et al., 2006; Dumontheil, Apperly, & Blakemore, 2010; Dumontheil, Houlton, Christoff, & Blakemore, 2010), as well as a more sophisticated understanding of more complex social hierarchies, structures and contexts. Adolescence is characterised by a greater sensitivity to social stimuli and social contexts, leading to the emergence of behaviours such as increased

emotional regulation (McRae et al., 2012), increased conformity and social "policing" of other people's behaviours in social situations (Steinberg & Morris, 2001). Finally, it is worth noting that certain elements of social cognition continue to develop into early adulthood. For example, the ability to recognise faces peaks in one's thirties (Susilo, Germine, & Duchaine, 2013).

1.2.2 Social cognitive development in atypical development

Children with neurodevelopmental disorders often display social cognitive impairments. The study of the trajectory of social cognition in typically developing children has been accompanied by an interest in the social cognitive trajectory of atypically developing children. A main focus has been on determining whether developmental stages are reached more slowly in atypically developing children, as well as in determining whether subtle impairments, or developmental delays are present prior to diagnosis. A full description of the trajectory of social cognition in atypically developing children is beyond the scope of this discussion, but has been extensively reviewed elsewhere (see Happe & Frith 2014 for a review). However, a few main points emerge from this research.

First, altered social cognitive development trajectories occur in many developmental disorders. Children with Down's syndrome display delays in the development of mutual gaze and eye contact. In contrast, infants with William's syndrome display abnormally long and intent looking at faces and eyes, particularly for unfamiliar faces (Tager-Flusberg, Plesa-Skwerer, Faja, & Joseph, 2003). Children with autism appear to display a largely typical social developmental trajectory until around 1-2 years of age, when there is a reduction in imitation, eye gaze, and social interest (Zwaigenbaum et al., 2005). Second, some evidence suggests that developmental delays in the acquisition of cognitive abilities can have "knock-on" effects on the acquisition of future cognitive skills, with impairments continuing to be observed in social cognition across later development (Dawson et al., 2004; Karmiloff-Smith,

1998). Lastly, while individuals with neurodevelopmental disorders often display an altered social cognitive developmental trajectory, impairments are rarely observable from birth (see Happe & Frith 2014).

1.2.3 Domain-specificity and domain-generality during development

1.2.3.1 Interactions between social domains

It is unclear to what extent different facets of social cognition rely on shared or distinct mechanisms. As a result, it is also unclear to what extent different domains of social cognition rely on one another during development (Happé et al., 2017). This is an important issue to address because it impacts on our understanding of social impairments in neurodevelopmental disorders; one account of social cognitive development views abilities acquired during early development as "building blocks" required for the development of more complex social processes. Under this "cascade" hypothesis, abnormal development of these early skills necessarily impairs the acquisition of later social abilities. "Cascade" models have been used to account for atypical social development (Johnson, Grossmann, & Kadosh, 2009). Whether or not this "cascade" model receives experimental support is unclear. Others have argued that there is some degree of independence between different social domains (Happé & Frith, 2014).

1.2.3.2 Social and non-social cognitive development

The extent to which social cognition relies on domain-specific versus domain-general mechanisms is still highly debated (see section 1.1.1). As a result, the relationship between the development of social cognition and non-social cognition is also not well understood.

At one extreme, social cognition could develop completely independently from other domains. In this case, abnormal development of social cognition would not affect the development of other abilities, and vice versa. At the other extreme, social and non-social

cognitive development could rely on completely shared mechanisms. In this circumstance, atypical development in one domain would be expected to be accompanied by equally pronounced abnormalities in the other domain.

Examining the altered trajectory of social development in neurodevelopmental disorders may provide insight into the processes underlying "typical" development. Individuals with neurodevelopmental disorders frequently display abnormal behaviours across multiple developmental domains. It has been unclear to what extent different symptoms are related to one another. Findings of "spared" abilities in the face of impairments in other domains suggest a degree of independence. For example, individuals with Down's syndrome have intellectual impairments but apparently spared social cognition (Rosner, Hodapp, Fidler, Sagun, & Dykens, 2004). Individuals with high-functioning autism display pronounced social difficulties but relatively intact non-social cognition. This has sometimes been taken as evidence of a partial or double dissociation between social and non-social cognition.

However, the interpretation of "spared" and "impaired" abilities in neurodevelopmental disorders is complicated (Karmiloff-Smith, 2009; Karmiloff-Smith et al., 2003). Sometimes, subtle impairments or developmental delays exist in apparently "spared" domains. These can be hard to detect because of both limitations in ability to test very young children, and because of the choice of controls, which may be matched for mental age. Atypically developing children may appear "spared" in one domain because they are performing as well as controls matched for mental age but who are younger. Compounding these difficulties in interpreting patterns of impairments is the fact that developmental insults may lead to considerable compensatory strategies. Hence, "spared" performance in one domain may be acquired by alternative strategies following disruption of the normal developmental trajectory of the domain.

In addition, not all evidence supports the notion that social cognition develops independently from non-social cognition. Multiple studies have found a positive relationship between executive function and theory of mind in both typically and atypically developing children (Carlson, Moses, & Breton, 2002; Hughes, 1998; Hughes & Ensor, 2007; Ozonoff, Pennington, & Rogers, 1991).

1.2.3.3 Social cognitive development and perceptual development

Perceptual development is another domain that may affect social cognitive development. Social interaction relies on sensory processing. We extract social meaning from sensory stimuli, which guides our social interactions. For example, we use facial expressions to interpret the emotions of other social agents.

Perceptual development has been proposed to play a role in shaping social development in typically and atypically developing children. Sensory symptoms are frequently observed in individuals with neurodevelopmental disorders. These sensory symptoms have been reported to precede social symptoms. Furthermore, multiple studies in autism spectrum disorders indicate that sensory symptoms predict both autism severity, and social symptoms including reduced play (Adamson, O'Hare, & Graham, 2006; Hilton, Graver, & LaVesser, 2007; Hilton et al., 2010; Kuhaneck & Britner, 2013; Liss, Saulnier, Fein, & Kinsbourne, 2006; Watson et al., 2011). However, the nature of the relationship between sensory symptoms and social dysfunction is unclear (Robertson & Baron-Cohen, 2017). Hence, the relationship between sensory development and social development requires further investigation.

It is worth noting that behavioural domains that are initially interdependent may become independent through experience-dependent development. In this scenario, the effect of a developmental insult on each domain would depend on the time in development at which the insult occurred, leading to different conclusions about domain-specificity and

interdependence (Karmiloff-Smith, 2009). As a result, determining the extent to which different domains of social cognition are interdependent or independent is a complicated endeavour.

In summary, much is still unknown about the relationship between social development and the development of other cognitive and perceptual abilities. Recent accounts of neurodevelopment, such as the "neurocontructivist" account, emphasise the need to give further thought to understanding how different cognitive domains interact during development. This approach views the acquisition of different skills not as an isolated process determined by either innate, genetic mechanisms, or environmental influences, but an interactive process in which different domains interact with one another and neural structures compete for representation of mental abilities (Karmiloff-Smith et al., 2003). This is likely to carry important implications for our understanding of atypical development in neurodevelopmental disorders, which typically present with altered behaviour across multiple developmental domains.

1.3 The "social brain"

1.3.1 Human neuroimaging and neuropsychology studies

Functional magnetic resonance BOLD imaging has been used to investigate the parts of the brain that subserve social cognition. These studies have revealed a network of brain regions that is highly active during social cognition, termed the "social brain". The brain regions that constitute the core "social brain" network are typically considered to consist of the fusiform face area (FFA), posterior superior temporal sulcus (pSTS), amygdala, temporo-parietal junction, medial and orbital prefrontal cortex, anterior cingulate cortex, and anterior temporal cortex (Brothers, 1996; Frith, 2007). The contribution of these brain regions to social cognition is reviewed briefly below.

1.3.1.1 The fusiform face area

The fusiform face area (FFA) is a region within the fusiform gyrus that is involved in the perception of faces. In humans, the FFA has been found to be disproportionately active during the viewing of faces relative to other stimuli including non-social objects, inverted or scrambled faces, and "face-like" non-face stimuli (Kanwisher et al., 1997). In addition, in vivo electrophysiological studies in monkeys demonstrate that neurons in the inferior and superior temporal cortex are responsive to faces (Hasselmo, Rolls, & Baylis, 1989; Perrett, Rolls, & Caan, 1982). Strong support for the notion that this region is involved in face recognition has been obtained from neuropsychology studies indicating that damage to the FFA causes prosopagnosia, a deficit in the ability to recognise familiar faces (Barton, Press, Keenan, & O'Connor, 2002).

1.3.1.2 The posterior superior temporal sulcus

The posterior superior temporal sulcus (pSTS) plays a role in processing biological motion and social gaze. The pSTS is highly active when two people are looking at each other (mutual gaze) (Pelphrey, Viola, & McCarthy, 2004), as well as during viewing of biological motion (Thompson, Clarke, Stewart, & Puce, 2005). Studies indicate that this region is more active during motion depicting complex social situations compared with animations of inanimate motion (Castelli et al., 2000; Schultz et al., 2003). It has also been suggested that the pSTS is involved in representing the intent of actions, on the basis that a portion of the pSTS appears sensitive to the consequences of biological motion on the surrounding environment (Saxe, Xiao, Kovacs, Perrett, & Kanwisher, 2004).

1.3.1.3 The amygdala

The amygdala is thought to be involved in emotion processing (Sergerie, Chochol, & Armony, 2008). The amygdala is highly active during tasks in which participants view or

make judgements about emotional facial expressions (Hariri et al., 2008). Lesions restricted to the amygdala are sufficient to impair facial expression perception (Adolphs, Tranel, Damasio, & Damasio, 1995). The amygdala appears to be particularly involved in the recognition of negative facial expressions. This has led to proposals that the amygdala processes the emotional saliency or relevance of stimuli during social cognition (Adolphs, 2010).

1.3.1.4 The temporo-parietal junction

Neuroimaging studies indicate that the temporo-parietal junction (TPJ) plays a role in "mentalising", the ability to make inferences about the mental states of social agents. Functional magnetic resonance imaging studies have consistently reported high levels of BOLD activity in the TPJ during theory of mind tasks. The TPJ is also significantly during active empathy tasks (Völlm et al., 2006). Whilst some have claimed that activity in the TPJ during theory of mind tasks merely reflects a more general role in orienting to salient stimuli (Corbetta & Shulman, 2002), neuropsychology studies provide more causal evidence for the role of the TPJ in mentalising. Indeed, transcranial magnetic stimulation of the TPJ leads to reduced mentalising during moral judgements (Young, Camprodon, Hauser, Pascual-Leone, & Saxe, 2010), and damage to the TPJ causes deficits in theory of mind (Samson, Apperly, Chiavarino, & Humphreys, 2004).

1.3.1.5 The medial/orbitofrontal cortex

The ventromedial/orbitofrontal cortex has been implicated in the recognition of social signals and selection of context-appropriate social behaviours. Damage to this region profoundly alters social behaviour and leads to "inappropriate" social responses (Barrash, Tranel, & Anderson, 2000; Eslinger & Damasio, 1985; Saver & Damasio, 1991). In neuroimaging studies, the medial and orbitofrontal prefrontal cortex have been found to be active during

"mentalising" tasks, as well during sarcasm detection, observation of violation of social norms, and in moral judgement tasks (Baron-Cohen et al., 1994; Berthoz, Armony, Blair, & Dolan, 2002; Moll et al., 2002; Uchiyama et al., 2006). The role of the orbitofrontal cortex in social cognition will be described in more detail in section 1.4.

1.3.1.6 The anterior cingulate cortex

Imaging tasks indicate a role of the anterior cingulate cortex (ACC) in the processing and feeling of emotions; the ACC is highly active during tasks inducing emotion, and during emotional recall (Bush, Luu, & Posner, 2000) and in tasks that involve processing negative social emotions, such as social rejection, disgust, and empathy for others' pain (Eisenberger, Lieberman, & Williams, 2003; Mataix-Cols et al., 2008; Singer et al., 2004). The ACC appears particularly active in tasks that evoke emotional conflict. For example, during facial emotion processing tasks, it is particularly active in conditions in which facial expressions and emotional words are incongruent (Etkin, Egner, Peraza, Kandel, & Hirsch, 2006). This is consistent with its proposed wider role in conflict detection (Yeung, Botvinick, & Cohen, 2004).

1.3.1.7 The anterior temporal cortex

The anterior temporal cortex has been found to play a role in the storage of social knowledge and moral concepts (Zahn et al., 2007; Zahn et al., 2017a; Zahn, Moll, Paiva, et al., 2009). The anterior temporal cortex is active during tasks involving the use of social concepts (Zahn et al., 2017a). Damage to the temporal poles impairs the ability to use social knowledge (Zahn et al., 2017a; Zahn, Moll, Iyengar, et al., 2009).

1.3.2 Investigations of social brain circuitry in rodent and primate models

The neural circuitry underlying social cognition has been probed through optogenetic, electrophysiological, and lesion methods in rodent models. The majority of the work has concentrated on the circuits involved in social approach and aggressive behaviours.

1.3.2.1 Neural circuits involved in social approach

Lesion studies in rodents and primates have indicated that the amygdala, cingulate cortex, and anterior cingulate cortex are involved in social approach and social motivation. Lesions to the ACC reduce social interest in other animals and social memory (Rudebeck et al., 2007). In monkeys, lesions of the cingulate cortex and amygdala have also been found to reduce social motivation and affiliative behaviour (Hadland, Rushworth, Gaffan, & Passingham, 2003; Machado & Bachevalier, 2006).

Optogenetic studies have provided further insight into the circuitry of social approach, revealing a network of brain regions involved in the control of social motivation and approach behaviours, including the basolateral amygdala, ventral hippocampus, medial prefrontal cortex and dorsal raphe nucleus. Reciprocal projections between the basolateral complex of the amygdala and ventral hippocampus have been found to regulate social approach. Activation of the projections from the basolateral amygdala to the ventral hippocampus reduces social approach and social interactions. Conversely, inhibiting this pathway increases social approach and social interactions (Felix-Ortiz & Tye, 2014). However, it is unclear whether the effect of activation of this pathway reflects a role in social motivation per se, or reflects a more general role in anxiety-related behaviours. Indeed, anxiety behaviours are also increased or decreased by activation and inactivation of this pathway, respectively (Felix-Ortiz & Tye, 2014). Hence, the effects of manipulation of this

pathway on social approach may reflect changes in anxiety levels, which may alter sociability.

Circuitry within the medial prefrontal cortex has also been implicated in social approach behaviours (Yizhar, Fenno, et al., 2011). Social approach after social defeat is increased by optogenetic activation of excitatory projections from the ventromedial prefrontal cortex/orbitofrontal cortex to the dorsal raphe nucleus (Challis, Beck, & Berton, 2014). A second study has implicated excitatory/inhibitory balance in the medial prefrontal cortex in regulating the duration of social interaction (Yizhar, Fenno, et al., 2011).

1.3.2.2 Neural circuits involved in aggression

Lesion studies in rodents and monkeys have consistently demonstrated that the orbitofrontal cortex plays a role in regulating aggression. Damage to the orbitofrontal cortex increases aggression in rodents and monkeys (De Bruin, Van Oyen, & Van De Poll, 1983; Machado & Bachevalier, 2006; Nonneman, Voigt, & Kolb, 1974).

Optogenetic investigations into the neural circuits mediating and controlling aggression have largely concentrated on downstream regions involved in effectuating aggression. Multiple studies indicate that the ventromedial hypothalamus plays a key role in the initiation of aggressive behaviour in rodents. Offensive aggression is enhanced by optogenetic activation of the ventromedial hypothalamus and reduced by inactivation of this region (Falkner, Grosenick, Davidson, Deisseroth, & Lin, 2016b). Optogenetic studies have revealed that defensive aggression is mediated by the dorsomedial division of the ventromedial hypothalamus, whereas aggression towards conspecifics is controlled by the ventrolateral ventromedial hypothalamus (Silva et al., 2013). Optogenetic activation of the ventromedial hypothalamus increases aggression both within the context of paradigms in which aggression is expected, and within contexts in which male mice do not normally display aggression

(Falkner et al., 2016b; Lin et al., 2011). In addition, the aggressive behaviours controlled by the ventromedial hypothalamus are inhibited by activation of a projection from the lateral septum to the ventromedial hypothalamus (Wong et al., 2016b).

Manipulation of the ventromedial hypothalamus and lateral septum is able to abort ongoing attacks, suggesting that these regions consist of "downstream" areas involved in producing aggressive behaviours. In contrast, optogenetic inhibition of the medial prefrontal cortex increases the probability of engaging in aggressive behaviours without suppressing ongoing attacks, suggesting that the medial prefrontal cortex acts as an "upstream" region that regulates aggression (Takahashi, Nagayasu, Nishitani, Kaneko, & Koide, 2014). Surprisingly, in this study, optogenetic activation of the orbitofrontal cortex did not affect aggression. This result contrasts with numerous studies indicating that the orbitofrontal cortex is involved in regulating aggression (De Bruin et al., 1983; Nonneman et al., 1974). However, the study did not examine the effects of inhibiting the orbitofrontal cortex on aggression, which may have a more substantive effect than activation of the orbitofrontal cortex. The authors also did not differentiate between different subregions of the orbitofrontal cortex, which have been found to have different functional roles (Elliott, Dolan, & Frith, 2000; Ongur & Price, 2000). In addition, it is likely that optogenetic light stimulation does not easily reach the orbitofrontal cortex.

Optogenetic studies have provided some insights into the circuitry underlying social cognition. However, there is still much that is unknown about how these circuits control social behaviour, and the individual contribution made by different brain regions. In addition, the majority of these studies have focussed on the circuitry underlying social approach and defensive aggression. Other aspects of social cognition have not received much investigation.

1.3.3 Neuromodulatory systems

1.3.3.1 Oxytocin

Oxytocin is a neuropeptide produced primarily in magnocellular neurons of the hypothalamus. Oxytocin projections are widespread across the brain, but are particularly pronounced in the nucleus accumbens, amygdala, lateral septum, hippocampus and brainstem/spinal cord. There is a strong projection from the hypothalamus to the posterior pituitary where oxytocin is released into the bloodstream.

Oxytocin has been linked to the production of a number of social behaviours. For example, oxytocin is known to play a role in maternal behaviour (Marlin, Mitre, D'amour, Chao, & Froemke, 2015) and is released during pregnancy and partuition. In virgin female mice, oxytocin mediates a rapid switch from displaying lack of interest in pups towards exhibiting maternal behaviours, such as nest building and pup retrieval (Pedersen, Prange, & Jr., 1979). The effect of oxytocin on pup retrieval occurs through release of oxytocin into the left auditory cortex (Marlin et al., 2015). These maternal behaviours are blocked by administration of an oxytocin antagonist (van Leengoed, Kerker, & Swanson, 1987). Similar results have been observed in prairie voles (Olazábal & Young, 2006).

In addition, oxytocin has also been implicated in affiliative behaviours and social recognition. Oxytocin and oxytocin receptor knock-out pups emit fewer "distress" vocalisations when separated from their mothers (Takayanagi et al., 2005; Winslow et al., 2000). Oxytocin is involved in the establishment of partner preferences in prairie voles (Insel & Hulihan, 1995). Adult oxytocin or oxytocin receptor knock-out mice display elevated aggression (Takayanagi et al., 2005; Winslow et al., 2000). Moreover, oxytocin and oxytocin receptor knock-out mice display deficits in social memory in the absence of pervasive deficits in social approach or olfaction (Crawley et al., 2007; Takayanagi et al., 2005). Administration of oxytocin into the

amygdala is sufficient to reverse deficits in social memory (Winslow & Insel, 2002). In addition, there is some evidence that oxytocin has similar effects in humans. For example, intranasal oxytocin has been found to improve recognition memory for angry and neutral faces (Savaskan, Ehrhardt, Schulz, Walter, & Schächinger, 2008) and increase trust (Kosfeld, Heinrichs, Zak, Fischbacher, & Fehr, 2005).

1.3.3.2 Serotonin

Serotonin (5-HT) has been particularly implicated in aggressive behaviour. Multiple lines of evidence associate impulsivity and aggression with 5-HT deficiency. Many of these studies are based on depleting 5-HT levels in the brain. This can be achieved quite easily because 5-HT is synthesized from the essential amino acid, L-tryptophan. Restricting L-tryptophan in the diet is sufficient to reduce the availability of 5-HT in the central nervous system. Acute tryptophan depletion increases state aggression and attention to aversive emotional stimuli (Cleare & Bond, 1995; Klaassen et al., 2002; Passamonti et al., 2012). In addition, fMRI studies indicate that tryptophan depletion reduces the functional connectivity between the amygdala and prefrontal cortex during processing of aversive social stimuli (Passamonti et al., 2012). Carriers of the MAOA-L allele, who display a greater tendency towards antisocial behaviours, are more vulnerable to this effect (Eisner et al., 2017). Neural activity during a go-no-go impulsivity task is significantly reduced in the orbitofrontal cortex, anterior cingulate and inferior temporal cortex after acute tryptophan depletion. Furthermore, the reduction in neural activity is greater in individuals with greater 5-HT depletion and greater trait impulsivity (Helmbold et al., 2015).

However, it is worth noting that there has been debate with regards to which monoamine oxidase A (MAOA) allele is associated with impulsivity and aggression, since the high-expression allele has been associated with impulsivity in healthy adult males (Manuck, Flory,

Ferrell, Mann, & Muldoon, 2000), but the low-expression allele has been associated with antisocial behaviour in individuals who suffered from childhood maltreatment (Caspi et al., 2002; Foley et al., 2004).

Rodent studies have also suggested a role of the 5-HT1 receptor class in regulating aggression and impulsivity. Mice with knock-down of the 5-HT1B receptor display increased aggression and impulsivity. Treatment with 5-HT1A or 5-HT1B agonists reduces aggression in mice and rats. Aggressive behaviours in mice with knock-out of the cannabinoid receptor 2 (CBR2) gene is accompanied by elevated COMT, MAO-A, 5-HTT and 5HT1B3 gene expression in the amygdala (Rodríguez-Arias et al., 2015). In socially isolated mice, aggressive behaviours are accompanied by elevated serotonin and dopamine levels, and can be reversed by diazepam, mGlu2/3 receptor agonists, and a 5-HT1A receptor agonist, supporting the role of 5-HT1 receptors in reducing aggressive behaviours (Ago et al., 2013).

1.3.3.3 Vasopressin

Vasopressin is a nonapeptide with an amino acid sequence that is strikingly similar to oxytocin, differing by only two amino acids (Stoop, 2012). Like oxytocin, vasopressin is synthesized by hypothalamic neurons that project to the brain and posterior pituitary. Vasopressin was first identified because of its antidiuretic action on the kidneys and, hence, is also known as antidiuretic hormone.

More recent evidence has implicated vasopressin in regulating aggression and pair-bonding. V1aR knock-out mice exhibit impaired social recognition and reduced anxiety (Bielsky, Hu, Szegda, Westphal, & Young, 2004). Vasopressin is released after bonding in male prairie voles, leading to formation of a pair-bond, an increase in aggression towards males, and paternal behaviour (Winslow, Hastings, Carter, Harbaugh, & Insel, 1993). The influence of vasopressin on paternal behaviour is thought to involve circuits within the amygdala, whilst

its role in pair bonding involves the nucleus accumbens, ventral pallidum and mediodorsal thalamus (Lim & Young, 2004). Some effects of vasopressin have also been reported in human studies. For example, during face perception tasks, vasopressin reduces perception of friendliness in males and increases feelings of affiliation in women (Thompson, George, Walton, Orr, & Benson, 2006).

1.3.4 Summary

In summary, there is a growing body of work at the human neuroimaging level investigating the network of brain regions involved in social cognition. This has been accompanied by some detailed work investigating the role of neuromodulators in social cognition in rodents. However, the cellular mechanisms underlying social cognition have been less extensively studied, and have focussed mainly on a smaller set of behaviours (social approach and aggression). Other aspects of social cognition have received less attention at the circuit level.

1.4 The orbitofrontal cortex and social cognition

1.4.1 The anatomy of the orbitofrontal cortex

1.4.1.1 Primate orbitofrontal cortex

The orbitofrontal cortex encompasses the most inferior and ventral portions of the prefrontal cortex. In humans, the orbitofrontal cortex is usually defined as the Brodmann areas 10, 11 and 47. Together with the medial prefrontal cortex, it is sometimes referred to as the "ventromedial prefrontal cortex". In non-human primates, the orbitofrontal cortex encompasses areas 10, 11, 12, 13 & 14. Areas 10 and 11 occupy the frontal pole and anterior orbital cortex. Areas 12, 13 and 14 consist of the lateral, medial, and ventromedial orbitofrontal cortex, respectively (Walker, 1940). The orbitofrontal cortex can be further subdivided into medial and lateral orbitofrontal cortex on the basis of differences in function and anatomical connections.

1.4.1.2 Rodent orbitofrontal cortex

Rodent prefrontal cortex is usually divided into the medial precentral (PrCm), prelimbic (PL), infralimbic (IL), and orbitofrontal cortices. More recently, several classifications have included the anterior cingulate cortex (ACC) in rodent prefrontal cortex. The orbitofrontal cortex is further subdivided into medial (MO), lateral (LO), ventral (VO) and ventrolateral (VLO) orbitofrontal cortex (Krettek & Price, 1977).

Rodent prefrontal cortex is far smaller, and less specialised than human and non-human primate prefrontal cortex. The extent to which rodents possess a prefrontal cortex has been the subject of extensive debate (Preuss, 1995; Uylings, Groenewegen, & Kolb, 2003). In Brodmann's original classification of cortical areas (1909), the prefrontal cortex was defined as the region rostral to the premotor areas possessing a prominent granular layer IV. The frontal lobe is largely agranular in rodents. Hence, Brodman's classification considered the prefrontal cortex to be unique to primates. Subsequently, Rose & Woolsey (1948) and Uylings et al (2003) argued that rodents possess a region analogous to human prefrontal cortex on the basis of similar projections from the thalamic mediodorsal nucleus, similarities in the effects of lesions, and the presence of reciprocal projections to monoaminergic brainstem nuclei.

The claim that the source of inputs from the thalamus can be used to define the prefrontal cortex adequately has been disputed (Preuss, 1995). This reignited the debate about whether rodents possess a prefrontal cortex that is homologous to primate prefrontal cortex (Preuss, 1995; Uylings et al., 2003). A key feature of the debate has centred around whether or not rodents possess an equivalent to the dorsolateral prefrontal cortex.

There is greater consensus that the rodent orbitofrontal cortex is homologous to human and non-human primate orbitofrontal cortex (Preuss, 1995). A region in the ventral and inferior

portions of the frontal lobe can be identified in both primates and humans. The analogy is also far simpler from the point of view of cytoarchitecture, since sections of primate orbitofrontal cortex are also agranular (Morecraft, Geula, & Mesulam, 1992). As a result, the presence of an orbital cortex in rats has been acknowledged even by those disputing that rodents possess an equivalent to the dorsolateral prefrontal cortex (Preuss, 1995). The analogy between rodent and primate orbitofrontal cortex is supported by similarities in anatomical connections, monoaminergic input, and function.

1.4.2 Anatomical connections of the orbitofrontal cortex

1.4.2.1 Afferent inputs to orbitofrontal cortex

The orbitofrontal cortex receives projections from a vast range of brain regions. This has been extensively studied in non-human primates. Projections to the orbitofrontal cortex include extensive input from all sensory modalities (Barbas, 1993). Multiple higher visual processing areas project to the (lateral) orbitofrontal cortex, including the inferior temporal cortex and superior temporal sulcus (Barbas, 1988; Morecraft et al., 1992). In addition, area TA in the superior temporal cortex sends auditory input to the orbitofrontal cortex (Barbas, 1993; Morecraft et al., 1992). Taste information is received from the anterior insula and frontal operculum (Baylis, Rolls, & Baylis, 1995; Rolls & Baylis, 1994a; Rolls, Sienkiewicz, & Yaxley, 1989). Area 11 receives projections from olfactory bulb, and area 47/12 receives somatosensory input from secondary somatosensory areas (Carmichael and Price, 1995a).

The orbitofrontal cortex also receives input from the amygdala, hippocampus and surrounding temporal lobe. Projections from the amygdala are particularly dense (Amaral and Price, 1984; Carmichael and Price, 1995a). These projections terminate most densely in upper layers 1 and 2 (Timbie & Barbas, 2014). Orbitofrontal cortex also receives projections from the hippocampus, parahippocampal areas, posterior cingulate, retrosplenial cortex,

entorhinal cortex and temporal pole. The midline thalamus, mediodorsal thalamic nucleus, insula and hypothalamus also project to orbitofrontal cortex.

In addition, the orbitofrontal cortex receives dense projections from other parts of the prefrontal cortex. Projections from the anterior cingulate cortex are particularly dense (García-Cabezas & Barbas, 2016). Lateral and medial prefrontal cortex, as well as premotor cortex, and to a lesser extent, periarcuate and frontal eye field, project to the orbitofrontal cortex (Barbas and Pandya, 1989; Carmichael and Price, 1995).

Whilst many early rodent studies do not differentiate between medial prefrontal cortex and orbitofrontal cortex projections, similar patterns of connections have been observed in rodent orbitofrontal cortex (Condé, Maire-lepoivre, Audinat, & Crépel, 1995; Krettek & Price, 1977; Mcdonald, 1991). In addition, these studies indicate that distinct subregions of rodent orbitofrontal cortex receive inputs from different brain regions. In the rat, medial orbitofrontal cortex receives input primarily from the anterior and posterior cingulate, mediodorsal thalamus, basolateral, postero-cortical and amygdalo-hippocampal subdivisions of the amygdala, medial prefrontal cortex, hippocampus, entorhinal cortex, and olfactory bulb. In contrast, ventral and lateral orbitofrontal cortex receive dense sensory input from visual, taste (insula), somatosensory, and olfactory (olfactory bulb and pirifrom cortex) areas, as well as posterior parietal cortex, mediodorsal thalamus, perirhinal cortex and basolateral amygdala (Reep, Corwin, & King, 1996).

1.4.2.2 Efferent projections of the orbitofrontal cortex

The orbitofrontal cortex projects to many areas of the brain. Topographically organised inputs are sent to the parahippocampal region. Medial, lateral, ventral and ventrolateral orbitofrontal cortex project to the perirhinal cortex and lateral entorhinal cortex. In contrast, projections to medial entorhinal cortex are primarily from the ventral orbitofrontal cortex

(Kondo & Witter, 2014). Orbitofrontal cortex also project to the spinal cord, dorsal raphe nucleus, dorsal and ventral striatum, basolateral nucleus of the amygdala, anterior and posterior lateral hypothalamus, ventral tegmental area and mediodorsal thalamus (Gabbott, Warner, Jays, Salway, & Busby, 2005; Rempel-Clower & Barbas, 1998).

1.4.3 The orbitofrontal cortex and non-social cognition

Studies investigating the role of the orbitofrontal cortex in non-social cognition indicate that this region plays a role in decision-making and goal-directed behaviour (Burke, Franz, Miller, & Schoenbaum, 2008; Rudebeck & Murray, 2014; Wallis, 2007). In humans, damage to the ventromedial/orbitofrontal cortex increases impulsive behaviour and decision-making under risk and uncertainty. For example, patients with orbitofrontal damage are impaired at selecting the most rewarding decks in the Iowa gambling task (Bechara, Damasio, Damasio, & Anderson, 1994). Similar impairments in decision-making have been observed in rodents and non-human primates with lesions to the orbitofrontal cortex. This includes impairments in the ability to extinguish or reverse a stimulus-reward association, deficits in making go/no-go discriminations, and impairments in the ability to select higher-value, delayed rewards over smaller-value, immediately available rewards (delay discounting) (Butter, McDonald, & Snyder, 1969; Izquierdo & Murray, 2005; McAlonan & Brown, 2003; Schoenbaum, Setlow, Saddoris, & Gallagher, 2003).

Multiple lines of evidence suggest that the orbitofrontal cortex plays a role in learning and representing the expected value of stimuli and outcomes. Electrophysiological and neuroimaging data demonstrate that orbitofrontal neurons are sensitive to features of sensory stimuli, such as stimulus identity and quality (Critchley & Rolls, 1996b; Tanabe, Iino, & Takagi, 1975). In particular, the orbitofrontal cortex appears to represent the value of sensory stimuli, such as the subjective pleasantness of taste (Kringelbach, O'Doherty, Rolls, & Andrews, 2003). This is also supported by *in vivo* electrophysiological recordings

demonstrating that neurons in the orbitofrontal cortex display selectivity to reward olfactory cues (Critchley & Rolls, 1996b).

The role of orbitofrontal neurons in representing expected value is also supported in decision-making tasks (Schoenbaum, Roesch, Stalnaker, & Takahashi, 2009). Animals with lesions to the orbitofrontal cortex fail to update choice preferences when a reward is devalued, e.g. by feeding the animal to satiety (Izquierdo & Murray, 2005; Machado & Bachevalier, 2006). Lesions to the orbitofrontal cortex also impair the ability to detect degradations in the contingency between actions and rewarding outcomes (contingency degradation) (Jackson, Horst, Pears, Robbins, & Roberts, 2016). In addition, *in vivo* electrophysiological recordings demonstrate that neurons in the orbitofrontal cortex fire in response to rewarding and aversive outcomes, as well as in anticipation of outcomes (Schoenbaum, Chiba, & Gallagher, 1999; Schoenbaum et al., 2003). Importantly, these studies indicate that the activity of neurons in the orbitofrontal cortex is better conceived as representing expected outcome, rather than a prediction error signal. Indeed, unlike striatal prediction error signals, orbitofrontal neurons continue to fire in response to reward even after learning, and do not alter firing when rewards are unexpected (Schoenbaum et al., 2003). Instead, responses of these neurons are modulated by the size of the reward, with stronger firing occurring in anticipation of preferred foods (Tremblay & Schultz, 2000). Similar results have been obtained in human neuroimaging studies; in a decision-making paradigm, BOLD activity in the orbitofrontal cortex correlated with goal and decision values, but did not represent prediction error (Hare, O'Doherty, Camerer, Schultz, & Rangel, 2008).

Evidence indicates that representations of the expected value of stimuli or outcomes in the orbitofrontal cortex are sensitive to context, such as the current goal, past action history and the value of alternative outcomes. For example, in tasks in which the absolute value of

conditioned stimulus stays fixed, but the value changes relative to the rewards obtained in previous trials, neurons in the orbitofrontal cortex rapidly update response to the stimulus as a function of prior reward history (Saez, Saez, Paton, Lau, & Salzman, 2017). Representations of task variables in the orbitofrontal cortex are also sensitive to the importance of these variables in the task (Schoenbaum & Eichenbaum, 1995a, 1995b). Moreover, orbitofrontal neurons respond differentially to objects based on the context they are in, and the reward value associated with the object in the given context (Farovik et al., 2015). As a result, it has been proposed that the orbitofrontal cortex functions as a "cognitive map" of task space, representing different task states, and the value of options according to current task state (Wilson, Takahashi, Schoenbaum, & Niv, 2014). According to this account, the orbitofrontal cortex integrates multisensory information that is relevant to task state, such as past memories, prior reward history, and the current value of stimuli and outcomes to determine what 'task state' we are in.

Studies of the role of the orbitofrontal cortex in non-social decision-making have also revealed that different regions of the orbitofrontal cortex make distinct contributions to decision-making. For example, in a probabilistic reversal learning task in rats, medial orbitofrontal lesions led to difficulty in the initial learning of action-outcome contingencies, reduced sensitivity to positive and negative feedback, and perseverative behaviour. In contrast, lateral orbitofrontal lesions impaired reversals, but did not cause perseverative behaviour, with particular deficits in win-stay/lose-shift strategies. As a result, it has been suggested that the medial orbitofrontal cortex is involved in identifying high probability reward and integrating goal values with action-outcome contingencies, whereas the lateral orbitofrontal cortex plays a role in maintaining choices when contingencies change (Noonan, Kolling, Walton, & Rushworth, 2012).

1.4.4 The role of the orbitofrontal cortex in social cognition

The orbitofrontal cortex is a key player in the "social brain" network (Adolphs, 2008). The importance of the orbitofrontal cortex for social cognition was first demonstrated in patient cases, which reported dramatic changes in interpersonal functioning and social cognition as a result of orbitofrontal damage, despite relatively intact cognition (Anderson et al., 2000; Barrash et al., 2000; Cicerone & Tanenbaum, 1997; Eslinger & Damasio, 1985; Saver & Damasio, 1991). These early studies suffered from limitations owing to small sample sizes, lack of control groups, and lack of differentiation between the orbitofrontal cortex and other portions of the medial prefrontal cortex. However, the findings of these case studies has been substantiated by larger-scale studies comparing groups of patients with damage to the orbitofrontal cortex to age-matched controls, or patients with brain damage in other regions (Berlin, Rolls, & Kischka, 2004; Grafman et al., 1996). Additional support comes from the finding that social abilities are related to the size of the orbitofrontal cortex in humans (Von Der Heide, Vyas, & Olson, 2014).

The orbitofrontal cortex is involved in multiple facets of social cognition. One function performed by the orbitofrontal cortex appears to be regulation of aggression. Studies indicate that patients with damage to the orbitofrontal cortex result in an increased tendency to behave aggressively and engage in angry outbursts, as well as difficulty controlling negative emotions (Anderson et al., 2000; Cicerone & Tanenbaum, 1997). Subsequent studies have supported the finding that these patients have difficulty self-regulating their behaviour and inferring emotional states (Beer, Heerey, Keltner, Scabini, & Knight, 2003). A role in the regulation of emotional responses is supported by an fMRI study in which participants viewed neutral or angry facial expressions before choosing a punishment for their opponent in a competitive reaction time task. Here, activity within the medial orbitofrontal cortex correlated negatively with aggressive behaviour (Beyer et al., 2015). Monkey and rodent

lesion studies also support this notion, as lesions to the orbitofrontal cortex result in heightened aggression (De Bruin et al., 1983; Machado & Bachevalier, 2006; Nonneman et al., 1974).

A second role of the orbitofrontal cortex appears to be in the representation of "social rules", and the selection of social behaviour that is appropriate to social context according to such rules. Patients with orbitofrontal damage struggle to differentiate socially "acceptable" and "inacceptable" behaviours, as measured by the social faux pas test (Lee et al., 2010). In addition, the orbitofrontal cortex is activated during reading violations of social norms (Bas-Hoogendam, van Steenbergen, Kreuk, van der Wee, & Westenberg, 2017). Studies of frontotemporal lobar degeneration also indicate that the orbitofrontal cortex is involved in representing the consequences of social actions, which may partially account for the difficulties patients display in selecting appropriate social behaviours (Zahn et al., 2017a). A role in the recognition of social contexts is also supported by rodent studies indicating that lesions to the orbitofrontal cortex impair the ability of rats to modulate play according to context (Pellis et al., 2006).

The orbitofrontal cortex also appears to be required for the recognition of social cues and signals. Individuals with orbitofrontal cortex damage display impairments of social perception, such as recognition of nonverbal social cues (Mah, Arnold, & Grafman, 2004), and impaired ability to judge facial expressions, especially those displaying negative emotions (Willis, Palermo, Burke, McGrillen, & Miller, 2010). In addition, anodal transcranial direct current stimulation (tDCS) to the orbitofrontal cortex has been found to enhance facial emotion recognition (Willis, Murphy, Ridley, & Vercammen, 2015).

Finally, studies indicate that the orbitofrontal cortex is involved in the ability to perform social judgements. Patients with orbitofrontal damage display impaired moral reasoning, and

difficulty judgements making social judgements (Mah, Arnold and Grafman, 2005; Ciaramelli et al., 2007a; Viskontas, Possin and Miller, 2007). This includes difficulty in judging the emotions and thoughts of other social agents. Patients with orbitofrontal damage display impaired theory of mind (Jenkins et al., 2014; Stone, Baron-Cohen, & Knight, 1998). Moreover, the orbitofrontal cortex is highly active during emotional and cognitive perspective-taking tasks (Hynes, Baird, & Grafton, 2006).

In summary, lesion and neuroimaging studies have revealed that the orbitofrontal cortex is an important structure for social cognition. However, orbitofrontal circuitry has not much been studied at the cellular level in relation to social cognition. Optogenetic studies have rarely focussed on the orbitofrontal cortex. Rodent models investigating impaired social cognition in the context of neurodevelopmental disorders have also rarely examined orbitofrontal cortex circuitry. Only one rodent model of neurodevelopmental disorders has conducted in vitro patch-clamp electrophysiology in the orbitofrontal cortex. This study revealed changes in excitatory circuitry in conjunction with social dysfunction in mice carrying a homozygous mutation in Dlgap2 (Jiang-Xie et al., 2014a). Collectively, these studies reveal that little is known about how the neural circuitry in orbitofrontal cortex contributes to social cognition.

1.5 Excitatory circuitry and social cognition

1.5.1 Excitatory circuitry and neurodevelopmental disorders

Numerous lines of evidence suggest that the development of excitatory circuitry may be disrupted in neurodevelopmental disorders. One line of support stems from genetic studies investigating risk variants for neurodevelopmental disorders. Indeed, multiple genes involved in synaptic function, development and regulation have been implicated in the aetiology of neurodevelopmental disorders such as autism and schizophrenia. Although some of these genes are expressed at both excitatory and inhibitory synapses, a large number of these genes

have been particularly linked to excitatory synapses (Pocklington, O'Donovan, & Owen, 2014). This is supported by the presence of excitatory circuitry deficits in multiple rodent models carrying genetic risk variants for neurodevelopmental disorders with a genetic component (Born et al., 2015; Dani & Nelson, 2009; Etherton, Blaiss, Powell, & Südhof, 2009a; Harrington et al., 2016; Jiang & Ehlers, 2013; Qiu, Anderson, Levitt, & Shepherd, 2011; Won et al., 2012).

Histology studies examining post-mortem brains of individuals with neurodevelopmental disorders also indicate that there may be changes in excitatory circuitry. In particular, these studies indicate that the formation and pruning of excitatory synapses may be altered in disorders of neurodevelopment. A number of studies have reported increased spine densities in the brains of individuals with autism, compared to age-matched controls (Hutsler & Zhang, 2010), and in the visual and temporal lobes of individuals with Fragile X syndrome (Irwin et al., 2001). In contrast, a reduction in the number of dendritic spines has been reported in deep layer 3 of prefrontal cortex and in the medial temporal lobe in schizophrenia (Garey et al., 1998; Glantz & Lewis, 2000; Harrison & Eastwood, 1998; Kolluri, Sun, Sampson, & Lewis, 2005).

Findings of altered plasma and serum glutamate levels in patients *in vivo*, and altered expression of proteins expressed at excitatory synapses in post-mortem brain tissue lend further support to the notion that excitatory circuitry is impaired in neurodevelopmental disorders (Harrison & Eastwood, 1998; Purcell, Jeon, Zimmerman, Blue, & Pevsner, 2001a; Shinohe et al., 2006). This has led to the proposal that neurodevelopmental disorders may be characterised by over or under-active synaptic pruning (Feinberg, 1982; Frith, 2004; Thomas, Davis, Karmiloff-Smith, Knowland, & Charman, 2016).

However, other research has instead implicated interneuron circuit dysfunction in the aetiology of neurodevelopmental disorders. Analyses of post-mortem tissue reveal a reduction in expression of the interneuron-specific marker, glutamic acid decarboxylase 67 (GAD-67) in layers 3-5 of dorsolateral prefrontal cortex in schizophrenic patients (Beasley and Reynolds 1997, Volk et al. 2000, Akbarian et al. 1995), as well as reduced expression of transcription factors that regulate interneuron development, such as brain-derived neurotrophic factor and tropomyosin receptor kinase B (Hashimoto et al. 2005, Weickert et al. 2003), and a reduced interneuron density (Benes, McSparren, Bird, SanGiovanni, & Vincent, 1991). Reduced GABA-A receptor density has also been observed in the anterior cingulate cortex in autism (Oblak, Gibbs, & Blatt, 2009). These studies point to a loss of inhibition, which may explain why some neurodevelopmental conditions are associated with an increased risk of epilepsy. It is likely that both inhibitory and excitatory circuitry disruptions can contribute to the pathophysiology of neurodevelopmental disorders. As a result, some researchers have proposed that neurodevelopmental disorders may result from a disruption of excitatory/inhibitory balance (Rubenstein & Merzenich, 2003).

1.5.2 Long and short-range brain connectivity and neurodevelopmental disorders

A recent proposal is that neurodevelopmental disorders are characterised by an imbalance between long and short-range connections, leading to altered integration of information. This has particularly been suggested in the context of autism spectrum disorders (Courchesne & Pierce, 2005). One popular formulation of this theory suggests that long-range connections are weaker and local connections are stronger in the brains of autistic individuals.

There is some evidence for reduced connectivity between brain regions in autism spectrum disorders. Autistic individuals display reduced functional connectivity between brain regions across a variety of behavioural tasks, including tasks assessing working memory, response

inhibition, emotion recognition, executive planning, sentence comprehension and visuomotor tasks (Just, Cherkassky, Keller, & Minshew, 2004; Kana, Keller, Minshew, & Just, 2007; Koshino et al., 2005; Villalobos, Mizuno, Dahl, Kemmotsu, & Müller, 2005; Welchew et al., 2005). These studies of functional connectivity implicate multiple brain regions, but reports of reduced connectivity are particularly common in the frontal lobe. However, this may reflect sample bias owing to the use of tasks that rely on the frontal lobes. Diffusion tractography imaging studies also lend support to the notion that long-range connections are reduced in autism spectrum disorders; reduced fractional anisotropy of white matter tracts has been reported in autistic individuals using tractography imaging (Frazier & Hardan, 2009). In particular, reduced white matter integrity has been reported in the corpus callosum (Alexander et al., 2007).

The evidence for increased local connectivity is weaker. One study reported increased fractional anisotropy, which suggests denser white matter, mainly concentrated in the frontal lobe in children with autism (Ben Bashat et al., 2007). Whilst this may indicate an increase in local connectivity, this evidence is highly indirect. In addition, multiple studies have reported opposite findings. Indeed, multiple studies observed reduced fractional anisotropy values in adolescents and adults with autism, particularly concentrated in the ventromedial/orbital prefrontal cortex, anterior cingulate cortex, and temporal lobes (Barnea-Goraly et al., 2004; Lee et al., 2007). Other studies report mixed findings, with some areas of increased and some areas of reduced fractional anisotropy (Ke et al., 2009).

The finding of increased dendritic spines in frontal, parietal, and temporal regions layer 2/3 has also been interpreted as indirect support for this hypothesis, because local connections are more prevalent than long-range connections (Courchesne & Pierce, 2005). However, dendritic spines in layer 2 and 3 consist of both local and long-range connections. An

increase in total number of dendritic spines could reflect an imbalance of short and long-range connections, or a global increase in the number of all connections. Furthermore, these studies provide no information about the strength of short and long-range connections, which could also be abnormal. It is also possible that changes in the number of connections occur to compensate for reduced synaptic strength of connections.

More recent studies have instead suggested that there both short and long-range connections may be reduced in autistic individuals. Indeed, some studies have found reduced functional connectivity both within and between brain regions in autistic individuals (Khan et al., 2013). It is also worth noting that some studies report increased long-range functional connectivity, most notably in cortico-subcortical connections (Mizuno, Villalobos, Davies, Dahl, & Müller, 2006).

Autism spectrum disorders are not the only neurodevelopmental disorder for which there is evidence that brain connectivity is altered. Reduced functional connectivity has also been reported in schizophrenia (Pachou et al., 2008). Moreover, abnormalities of the corpus callosum have been found in William's syndrome and schizophrenia (Narr et al., 2000; Tomaiuolo et al., 2002).

Collectively, these studies support the idea that brain connectivity is abnormal in disorders of neurodevelopment. However, it has proven difficult to ascertain the nature of the abnormalities. One potential problem is that there is likely to be considerable variation within and across conditions due to symptom and aetiological heterogeneity. It is likely that different subgroups of patients may present with different abnormalities. Moreover, the methods used to investigate connectivity in individuals with neurodevelopmental disorders are indirect and difficult to interpret. For example, changes in fractional anisotropy can reflect myelination, density of axons or changes in axonal diameter (Zatorre, Fields, & Johansen-Berg, 2012).

Functional connectivity is also difficult to interpret, as it is unclear to what extent functional connectivity reflects structural connections (Leopold & Maier, 2012). In addition, these techniques do not probe the strength and timing of connections, which could also be altered. Invasive techniques combining structural and functional electrophysiological investigation are required in order to properly examine the integration of short and long-range connections.

1.5.3 Maturation of excitatory circuitry and social cognition

Excitatory circuits can be altered by social experience during sensitive periods of social development. This has predominantly been studied in rodents during the "juvenile play period", a period of rodent social development from around P21 - P36 during which experience is vital for shaping later social behaviour. Immunohistochemical investigations suggest that social isolation during development may alter the properties of excitatory circuitry. However, the direction of change has not always been consistent. Multiple studies have reported reduced expression of markers of excitatory synapses, including synapsin 1, PSD-95, GluR1 and NR1 after social isolation (Hermes, Li, Duman, & Duman, 2011; Y. Zhang et al., 2012). More recently, however, increased mRNA expression of presynaptic excitatory (glutamate) markers vGlut1, EAAT1 and GLS1 was found in mouse medial prefrontal cortex after social isolation from P38, with no change in expression of inhibitory or myelin markers (Lander, Linder-Shacham, & Gaisler-Salomon, 2017). Differences in results may stem from differences in the time period during which social isolation occurred, as well as differences on the effect of social experience on different brain regions.

Histological studies of dendritic spines also support the notion that social experience can modify excitatory circuitry, and that effects may depend on the brain region investigated. In one study, 8 weeks of social isolation from P21 onwards resulted in a reduction in the number of dendritic spines in the hippocampus and medial prefrontal cortex of rats (Silva-Gómez, Rojas, Juárez, & Flores, 2003). Increased spine density, dendritic length and dendritic

complexity of pyramidal neurons has also been reported in the anterior cingulate cortex of rats isolated from P21 (Bock, Murmu, Ferdman, Leshem, & Braun, 2008). In contrast, social isolation leads to reduced apical dendritic length and complexity in the orbitofrontal cortex (Bock, Murmu, Ferdman, Leshem, & Braun, 2008), whilst experience with a greater number of social partners during development leads to more complex dendritic branching of pyramidal neurons in rat orbitofrontal cortex. However, histological studies have also suffered from inconsistent results. One study reported increased complexity of dendritic branching in the medial prefrontal cortex after social isolation during development, which is at odds with the results found by Silva-Gomez et al 2003 (Bell, Pellis, & Kolb, 2010a). Moreover, a different study found an inverse correlation between social behaviours and apical dendrite length in the orbitofrontal cortex (Ferdman et al., 2007), which contrasts with the results reported by Bock et al 2008. One possible explanation for discrepancies in findings may be differences in the age at which rat brains were examined after testing.

In summary, rodent studies indicate that social experience may shape the post-natal development of excitatory circuitry in the prefrontal cortex. However, the manner in which social experience affects excitatory circuitry development remains unclear. In addition, studies so far have concentrated on structural properties of excitatory synapses. It is possible that the functional properties of excitatory synapses are also modified by social experience.

1.5.4 Long-range connectivity and social cognition

Multiple studies indicate that abnormal long-range connectivity may contribute to impaired social cognition. In one functional connectivity study, mice socially isolated during adolescence were found to display abnormal connectomes, including significantly increased connectivity in right dorsolateral orbitofrontal cortex, entorhinal cortex and perirhinal cortex. Notably, the greatest abnormalities were concentrated around the right dorsolateral

orbitofrontal cortex, with increased connectivity between regions, and reduced modularity within the orbitofrontal cortex (Liu et al., 2016).

Altered long-range connectivity has also been reported in other mouse models displaying social impairment. For example, mice with reduced microglia during the early postnatal period as a result of knockout of the mouse chemokine receptor, *Cx3cr1*, display reduced functional connectivity between the dorsal hippocampus and the medial prefrontal cortex, in conjunction with diminished social motivation (Zhan et al., 2014). Moreover, mice with deficient immunity display both reduced social interaction, and hyper-connectivity between prefrontal and insular regions (Filiano et al., 2016).

Myelination in the prefrontal cortex may be particularly important for social cognitive development. Social isolation for 2 weeks after weaning in mice alters oligodendrocyte morphology and myelin thickness in the medial prefrontal cortex, without affecting oligodendrocyte density (Liu et al., 2012; Makinodan et al., 2016; Makinodan, Rosen, Ito, & Corfas, 2012). Treatment with clemastine in the medial prefrontal cortex can reverse social avoidance in socially isolated mice if mice are isolated from adulthood only (Liu et al., 2016a), but not if social isolation occurs during the P21-P35 period (Makinodan et al., 2012). The notion that changes in white matter support social development is corroborated by human imaging studies demonstrating increases in white matter fractional anisotropy during adolescence, which is an important period of social development (Barnea-Goraly et al., 2005; Choudhury et al., 2006).

1.6 Rodent models of neurodevelopmental disorders

1.6.1 What are rodent models good for?

Studies of neurological basis of neurodevelopmental/psychiatric disorders in humans suffer from limitations. First, only non-invasive techniques can be used, such as neuroimaging, or

histological measures from post-mortem tissue. These provide only limited insight into cellular and molecular mechanisms. Second, there is considerable heterogeneity across individuals with the same condition. This includes heterogeneity both in symptoms and in aetiology. Neurodevelopmental and psychiatric disorders are complex disorders that typically include a genetic and an environmental component. Different individuals with the same condition can present with completely non-overlapping sets of symptoms. Different individuals can also carry distinct genetic and environmental risk factors for disease. Even in individuals carrying the same genetic mutations, there will be variability as a result of different genomes and different environmental factors. This variability makes it difficult to identify cellular and molecular mechanisms of disease.

Rodent models offer a possibility to address some of these limitations. Rodent models offer a "way in" to studying the cellular and molecular mechanisms in more detail with *in vivo* and *in vitro* techniques. Rodent models also offer the advantage that it is possible to study single genetic or environmental factors in isolation, with other genetic and environmental background factors held constant. Rats and mice possess many similarities with humans in terms of genetics and environments. The mouse genome has more than 99% similarity to the human genome, making it a good tool to study the effect of genetic mutations. Since rats and mice are commensal to humans, they have also encountered similar environments as humans, and may have been shaped in similar ways by the environment.

However, rodent models suffer from limitations and there are many challenges in developing good rodent models of neurodevelopmental and psychiatric disorders. Diagnosis in psychiatry relies on subjective assessment of symptoms, rather than objective tests of biological markers. Hence, the face validity of a rodent model in representing different symptoms or behaviours is important (Chadman, Yang, & Crawley, 2009). However, it is

not clear how translatable mouse behaviours are to human symptoms. Rodents are not small humans, and certain behaviours may be species-specific. Hence, some symptoms may be unable to be modelled in rodents. For example, language impairments cannot be modelled in rodents.

A second issue stems from the heterogeneity of psychiatric and neurodevelopmental disorders. The aetiology of psychiatric and neurodevelopmental disorders is complex, typically resulting from genetic, environmental, and epigenetic factors. The genetics itself are also complex, resulting from a combination of low-risk common variants, and rarer, higher-risk variants (Manolio et al., 2009). Variability is also present at the level of phenotype; it is possible for two individuals to have completely non-overlapping symptoms, despite sharing the same diagnosis. Likewise, it is possible for symptoms to overlap in two individuals diagnosed with different conditions. As a result, it is unlikely that a single rodent model could encapsulate all features of a condition.

Issues relating to the complex aetiology of neurodevelopmental and psychiatric conditions impact on the question of the construct validity of rodent models (Chadman et al., 2009). This refers to the extent to which a rodent model recapitulates the aetiological processes e.g. genetic or environmental that contribute to a disorder. The extent to which a rodent model possesses construct validity is unclear in the case of complex neurodevelopmental and psychiatric conditions, where genetic risk factors possess incomplete penetrance, are heterogeneous across individuals, and display overlap across conditions (Nestler & Hyman, 2010).

As a result, it is recognised that not all features of a condition can be encapsulated using a single mouse model. Instead, different models may be used to investigate different components of a condition. One approach to translating findings from mouse models back to

humans is the "endophenotype" approach, which moves away from a focus on individual symptoms towards a focus on stable phenotypes that have a clear genetic connection (Gould & Gottesman, 2006). These phenotypes can be either behavioural or biological. In addition, multiple researchers have argued for a move away from focussing on modelling symptom clusters and individual conditions, towards a focus on determining how specific neural circuits contribute to distinct behaviours that cross clinical boundaries (Insel & Cuthbert, 2015).

1.6.2 Rodent models of social cognition

Human social cognition is highly sophisticated. It incorporates cultural values and relies on language. Some have argued that aspects of social cognition are "uniquely human" and that our social cognitive abilities far exceed those of other animals (Saxe, 2006). What does this mean for modelling of social cognition in rodents?

Whether or not non-human animals display more complex social abilities such as theory of mind or empathy has been much debated and is unclear (Ben-Ami Bartal, Decety, & Mason, 2011; Heyes, 1993; Silberberg et al., 2014). However, rodents are social animals that engage in high levels social behaviours. These behaviours include reciprocal social interactions, sexual behaviour, aggressive behaviours, scent marking, parenting behaviours and communal nest building, as well as juvenile play behaviours.

1.6.2.1 Reciprocal social interactions

Reciprocal interactions between pairs or groups of rodents can be measured by placing animals in a testing arena or cage and recording their interactions (Bolivar, Walters, & Phoenix, 2007). Multiple social behaviours can be scored in terms of their duration and number. This includes measurement of sniffing (e.g. nose-to-nose, anogenital, and body

sniffing), following/chasing, crawling under/over, mounting and wrestling, as well as measures of aggressive behaviours.

In juvenile animals, juvenile play behaviours can also be assessed. Juvenile and adolescent rodents engage in play behaviours, and are highly rewarding (Panksepp, 1981; Trezza, Baarendse, & Vanderschuren, 2010). Rats engage in higher levels of juvenile play than mice. To investigate juvenile play, the frequency and duration of play behaviours, such as pinning, pouncing, boxing and wrestling is typically measured. A more complete description of the functions of juvenile play is provided in chapter 4.

Social interaction measures provide insight into the social behaviours of test rodents but suffer from limitations. First, the behavioural measures are fairly non-specific. If interaction is altered, it is difficult to ascertain the underlying deficit. For example, does the change in behaviour reflect a change in social motivation, or an inability to perform appropriate social responses? Second, we possess limited insight into the structure of "normal" social interactions in mice. Thus, we are limited to quantitative measures rather than being able to tell if interactions differ in quality. Limited understanding of rodent social behaviours also feeds into the difficulty in determining the nature of changes in social interactions. Third, social interaction is reciprocal and the behaviour of one animal is likely to be impacted by the behaviour of the other. Thus the interpretation of the behaviour of one animal is confounded by the reaction of the other animal.

1.6.2.2 Social approach

The three-chamber social approach task provides a way to measure social motivation (i.e. the tendency to engage in social approach) independently of other facets of social behaviour, or of the behaviour of the partner animal (Moy et al., 2009). Here, the test animal is placed in a three-chambered box comprising an atrium which allows access to two side chambers with

wire cups in them. One side chamber contains a partner conspecific trapped in the wire cup. The wire cup in the other side chamber either contains an object or is left empty. Preference for social stimuli over non-social stimuli is measured by comparing time spent in either side chambers. This provides a clearer measure of social motivation. However, it may be confounded by issues such as anxiety (animals that are more anxious may be more likely to avoid social contact).

1.6.2.3 Social preference tests

In these tests, rodents are given the choice between different partners. This can be conducted in a three-chamber or a Y maze using the two side arms. This can be used to determine whether rodents can differentiate between social partners with different characteristics. For example, social recognition memory can be tested by measuring preference for a novel partner over a familiar partner (McFarlane et al., 2008).

1.6.2.4 Resident-intruder tasks

This task is used to assess aggression by placing one rodent in the cage of another rodent. This can be used to measure both offensive and defensive aggression between the "intruder" animal and the "resident" animals (Koolhaas et al., 2013).

1.6.2.5 Olfactory pheromones

Olfactory cues act as a key communicative signal in rodents. One source of olfactory cue consists of urinary deposits. These carry information about the characteristics of an animal (for example, sex) and elicit high levels of investigation from other mice (Arakawa, Blanchard, Arakawa, & Dunlap, 2008). The interest in social urinary odours exceeds levels of interest towards non-social odours. The duration and number of sniffing bouts elicited from different urinary deposits can be used as a marker of social motivation. In addition, repeated

presentation of a urinary odour followed by the presentation of a novel odour can be used to measure recognition of social odours using an olfactory habituation/dishabituation paradigm (Yang, Crawley, Yang, & Crawley, 2009). In addition, urinary deposits also function as territorial scent marks. The number of scent marks made in proximity to an olfactory cue can be used as a measure of social motivation and olfactory communication (Arakawa et al., 2008).

1.6.2.6 Ultrasonic vocalisations

High-frequency complex ultrasonic vocalisations are a second for of communicative signal used by rodents. These can be captured by sensitive microphones and advanced software designated to capture sounds in the ultrasonic range. Ultrasonic vocalisations are emitted in a range of social situations, including by pups upon separation from the nest, by juvenile animals during social interactions, by males upon presentation of female urine or during social interaction with a female, and by female mice in a resident-intruder paradigm (Branchi, Santucci, & Alleva, 2001; Holy & Guo, 2005; Knutson, Burgdorf, & Panksepp, 1998a, 2002; Moles, Costantini, Garbugino, Zanettini, & D'Amato, 2007).

However, the structure and meaning of ultrasonic vocalisation calls is still largely unknown. It is unclear how ultrasonic vocalisations map onto different social situations or behaviours. Some broad divisions can be recognised. For example, researchers distinguish between lower frequency "flat" vocalisations which cluster around the 25 kHz range, and higher-frequency vocalisations that can range from 30-80 kHz and average around 50 kHz. The latter has been found to be predominantly emitted during aversive situations, whilst the former occur during rewarding situations.

1.7 Aims

The aim of this thesis is to examine the contribution of excitatory circuitry within the orbitofrontal cortex to social cognition using a combination of behavioural testing and *in vitro* single-cell patch-clamp electrophysiology.

I begin by investigating the contribution of orbitofrontal circuitry to social dysfunction in a mouse model carrying a genetic deletion that is a risk factor for multiple neurodevelopmental disorders (chapters 2- 3). Previous experiments have demonstrated that these mice display impaired social behaviour and may be a good model of impaired social cognition (Grayton, Missler, Collier, & Fernandes, 2013). In chapter 2, I seek to assess the validity of this mouse line as a model of social dysfunction and to further characterise this phenotype. In chapter 3, I investigate whether abnormal social behaviour was accompanied by disrupted excitatory circuitry within the orbitofrontal cortex using single-cell patch-clamp electrophysiology in orbitofrontal cortex brain slices.

The second part of this thesis investigates the role of sensory experience in shaping the development of social cognition and the orbitofrontal cortex (chapters 4-5). This is achieved by depriving rodents of whisker input during a sensitive period of social development. Chapter 4 concentrates on the effect of this manipulation on social development. Finally, in chapter 5, I investigate the impact of sensory deprivation on the development of "social brain" circuitry in the orbitofrontal cortex.

2 Abnormal social behaviour in the *Nrxn1α* KO mouse model of neurodevelopmental disorders

2.1 Introduction

2.1.1 Social cognition in neurodevelopmental disorders

Impaired social cognition is a feature of many neurodevelopmental disorders (see section 1.1.3). For instance, abnormal social behaviour is one of the diagnostic features of autism spectrum disorders (DSM-V). Impaired social cognition is also recognised as an important feature of schizophrenia, although it is not a diagnostic feature of the condition (Penn et al., 1997; Penn, Sanna & Roberts, 2008; Green & Horan, 2010). In addition, altered social cognition has been observed in other neurodevelopmental conditions possessing a strong genetic component, such as William's syndrome, Fragile X syndrome, and Turner's syndrome (Bellugi et al., 2000; McCauley et al., 1987; Laws & Bishop, 2004; Cornish et al., 2005).

There are both similarities and differences in the profile of impaired social cognition across neurodevelopmental disorders. Similarities in deficits have been reported across numerous facets of social cognition. Impaired theory of mind has been observed in autism spectrum disorders, schizophrenia, and William's syndrome (Baron-Cohen et al., 1985; Bora, Yucel, & Pantelis, 2009; Frith & Corcoran, 1996b; Perner, Frith, Leslie, & Leekam, 1989; Porter, Coltheart, & Langdon, 2008; Tager-Flusberg & Sullivan, 2000). Difficulties with joint attention and pointing are present in children with autism spectrum disorders, as well as some children with William's syndrome (Mundy et al., 1986; Baron-Cohen, 1989; Lincoln et al., 2007). In addition, there are similar difficulties with emotion recognition and perception of social cues in autism spectrum disorders, William's syndrome, Turner's syndrome and

schizophrenia (Adolphs, Sears, & Piven, 2001; McCauley et al., 1987; Brune, 2005b; Lacroix, Guidetti, Rogé, & Reilly, 2009; Loveland et al., 1995; Walker, Marwit, & Emory, 1980). Individuals with autism spectrum disorders struggle to read emotions from faces and eyes, and make inappropriate judgements of trustworthiness from faces (Adolphs et al., 2001) and are poorer at interpreting emotions (Loveland et al., 1995). Individuals with schizophrenia are impaired also at emotion recognition (Brune, 2005; Walker et al., 1980). Difficulties interpreting facial expressions have also been reported in William's syndrome (Lacroix et al., 2009; Meyer-Lindenberg et al., 2005; Plesa-Skwerer et al., 2006). Finally, "inappropriate" behaviour, aggressive outbursts and disinhibition have been described in individuals with autism spectrum disorders, schizophrenia, William's syndrome, and Prader-Willi syndrome (Dimitropoulos, Ho, Klaiman, Koenig, & Schultz, 2009; Einfeld et al., 1997; Einfeld, Tonge, Turner, Parmenter, & Smith, 1999; Hoptman et al., 2010). For example, individuals with autism spectrum disorders often engage in "inappropriate" responses to social situations, such as engaging in embarrassing behaviours in public, or aggression (Bartak & Rutter, 1976). Individuals with autism spectrum disorders, and Prader-Willi syndrome both display behavioural difficulties and disinhibition (Dimitropoulos et al., 2009; Einfeld et al., 1999) Likewise, individuals with William's syndrome also display "inappropriate" social behaviour, such as inappropriate trustworthiness of strangers, disinhibition and behavioural problems (Einfeld et al., 1997).

There are also notable differences between the social profiles of individuals across different neurodevelopmental disorders. For example, individuals with autism spectrum disorders often display reduced motivation to engage in social interaction (Chevallier, Kohls, Troiani, Brodkin, et al., 2012), and individuals with schizophrenia display social withdrawal (Blanchard, Bellack, & Mueser, 1994). In contrast, children diagnosed with William's syndrome are hypersociable (Dodd & Porter, 2010; Jones et al., 2000). In addition,

individuals with autism spectrum disorders tend to show reduced preference for looking at faces and engaging in eye contact (Mundy et al., 1986), whereas individuals with William's syndrome display increased interest in faces and eyes than controls (Riby & Hancock, 2008; Riby & Hancock, 2009; Riby & Hancock, 2009).

Importantly, impairments in social cognition in neurodevelopmental disorders are also characterised by variability within individuals with the same condition. This is particularly notable in the case of social motivation in individuals with autism spectrum disorders. Many individuals with autism spectrum disorders display reduced attention to social stimuli and lack of interest in social interaction are often reported in ASDs (Baron-Cohen & Wheelwright, 2003; Leekam & Ramsden, 2006; Liebal, Colombi, Rogers, Warneken, & Tomasello, 2008; Osterling, Dawson, & Munson, 2002). However, this is not the case for all individuals with autism spectrum disorders. Whilst some children appear disinterested and "socially aloof", or engage passively in social interaction, others display heightened, persistent motivation to interact socially in a manner that is "odd" and leads to social rejection (Wing & Gould, 1979). This is more similar to the "hypersociability" described in individuals with William's syndrome (Davies, Udwin, & Howlin, 1998; Jones et al., 2000).

This variability in symptoms has important implications for attempts to understand the genetic, molecular and cellular mechanisms underlying impaired social cognition in neurodevelopmental disorders. Rather than considering impaired social cognition as a single uniform construct, it is important to remember that individuals can present with distinct social profiles. Different social cognitive impairments may arise from disruption of distinct neural mechanisms. Hence, it is important to understand how neural circuitry gives rise to different aspects of social cognition, and to ensure that all aspects of impaired social cognition are given attention at the behavioural, cognitive, and neural levels.

2.1.2 Cognitive deficits and neurodevelopmental disorders

Cognitive deficits are common in individuals with neurodevelopmental disorders. The extent of these cognitive impairments can vary widely. Many individuals with neurodevelopmental disorders display intellectual disability (ID) characterised by pervasive deficits in learning, memory, language and speech. For example, comorbid intellectual disability is present in around 50-70% of individuals with ASDs, and around 5% of individuals with schizophrenia (Fombonne, 2003; Morgan, Leonard, Bourke, & Jablensky, 2008a). In addition, cognitive deficits are apparent in many individuals who do not meet the criteria for intellectual disability. For instance, cognitive deficits are observed in first-episode patients with schizophrenia who have not yet received any treatment (Mohamed et al., 1999). Cognitive impairments are also seen in individuals with autism spectrum disorders (Rutter, 1983). Difficulties with executive function, such as impairments in attention, working memory, and problem-solving, are particularly common (Green et al., 2004; Hughes, Russell, & Robbins, 1994).

The relationship between social and non-social cognition has been the subject of much debate. The structure of social cognition is largely unknown (see Happe & Frith 2014). Some have argued that we possess "specialised", modular cognitive and neural processes for social cognition that are distinct from those engaged during non-social cognitive processes (Brothers, 1996). In contrast, others have maintained that social and non-social cognition rely on shared cognitive and neural resources (Heyes, 2012).

As a result, the relationship between social impairments and non-social cognitive deficits in individuals with neurodevelopmental disorders is unclear. There has been debate with regards to whether impairments in social and non-social cognition are related, and specifically, whether or not impairments in one domain are secondary to the other. One account of social impairments in individuals with neurodevelopmental disorders such as autism proposes that

these impairments occur as a result of cognitive deficits. In particular, researchers have suggested that impairments in theory of mind arise due to deficits in executive function, or language (Ozonoff, Pennington, & Rogers, 1991; Joseph & Tager-Flusberg, 2004). The notion that social cognitive impairments are caused by cognitive deficits has received some support. The risk of autism spectrum disorder increases with lower IQ (Wing & Gould, 1979), and IQ is a strong predictor of psychosocial outcome in individuals with autism (Rutter, 1970). In addition, there is a relationship between executive function and theory of mind in developing children (Ozonoff & McEvoy, 1994). However, the presence of a relationship does not imply causality; a different account is that deficits in executive function are secondary to social impairments in processes such as theory of mind. Indeed, a separate study found that while theory of mind and executive function were highly related in autistic children, impaired theory of mind was observed in the absence of executive dysfunction in some children, whilst the converse was not seen (Pellicano, 2007).

However, other lines of research suggest that social and non-social cognitive deficits are separate and arise from distinct mechanisms. Multiple studies have failed to find a relationship between cognitive and social cognitive symptoms in individuals with neurodevelopmental disorders. Twin studies investigating the heritability of these symptoms likewise suggest that social and non-social cognitive symptoms arise from largely non-overlapping genetic factors (Ronald, Happe, & Plomin, 2005; Ronald et al., 2006). In addition, there is evidence at the neural level suggesting that social and non-social cognition rely on distinct mechanisms; some brain regions appear to be preferentially involved in processing social stimuli (Kanwisher et al., 1997; Saygin, 2007). Lastly, there is evidence of "double dissociations" between social and non-social cognition in individuals with neurodevelopmental disorders. For example individuals with Down's syndrome have intellectual impairments with an apparent sparing of social cognition (Rosner et al., 2004),

whereas high-functioning individuals with ASD display abnormal social behaviour in the absence of prominent cognitive deficits (Baron-Cohen et al., 1997, 1985). However, questions have been raised as to whether or not "spared" abilities are truly intact in these conditions, and the legitimacy of invoking "double dissociations" in the context of neurodevelopment has been questioned (Karmiloff-Smith et al., 2012; Karmiloff-Smith, Scerif, & Ansari, 2003).

2.1.3 Genetics and neurodevelopmental disorders

Human diseases vary in their genetic architecture. Simple Mendelian disorders are caused by a single genetic mutation and display a clear pattern of inheritance. In other conditions, multiple genetic factors can contribute to risk for disease, and there is no clear-cut pattern of inheritance. These are called complex, or multifactorial, conditions. In these conditions, both rare, moderate-risk and common, low-risk variants in multiple genes, and environmental factors may contribute risk for disease.

Genetics plays a considerable role in the majority of neurodevelopmental disorders. Some neurodevelopmental disorders are caused by known genetic abnormalities. For example, Down's syndrome is caused by the possession of a third copy of the genes on chromosome 21, and Fragile X syndrome is caused by an increase in trinucleotide repeats on the FMR1 gene (Lejeune, Gautier, & Turpin, 1959; Verkerk et al., 1991). However, other neurodevelopmental conditions such as autism spectrum disorders and schizophrenia also possess a considerable genetic component. Twin studies indicate heritability estimates between 56-95% for autism spectrum disorders (Bailey et al., 1995; Colvert et al., 2015). Heritability estimates of 40-80% have been found for schizophrenia (Sullivan, Kendler, & Neale, 2003b). The importance of genetics in conditions like autism is supported by the finding that non-affected family members often display subclinical features (Losh et al., 2009).

Although monogenic causes can be found in about 5 % of individuals with autism spectrum disorders (Abrahams & Geschwind, 2008), the genetic architecture of autism spectrum disorders and schizophrenia is complex; multiple genetic variants may contribute risk for the conditions, and environmental and epigenetic factors may also play a role (Manolio et al., 2009). This complex genetic architecture poses a considerable challenge for understanding the genetic aetiology of these neurodevelopmental disorders. Difficulty elucidating the genetics of neurodevelopmental and psychiatric conditions is compounded by the fact that there is both locus and allelic heterogeneity in the genetic factors contributing to risk. In addition, there is considerable genetic overlap across neurodevelopmental and psychiatric conditions (Crespi, Stead, & Elliot, 2010).

One approach to elucidating the genetic aetiology of complex disease assumes that common conditions are caused by a number of common variants that each explain several percent of the risk (the "common disease-common variant" approach) (Lohmueller et al., 2003; Pritchard, 2001; Reich and Lander, 2001). Initial searches for common risk variants using genome-wide association (GWA) studies had mixed success. Common, low-risk variants have been identified in schizophrenia (Maher, 2008; Manolio et al., 2009), but attempts have proven less successful for autism spectrum disorders (Anney et al., 2012). More recently, progress has been made in identifying common genetic risk factors for psychiatric diseases in the form of small nucleotide polymorphisms (SNPs). These consist of common DNA sequence variations in coding and non-coding regions of the DNA. Over 108 SNPs have been identified as risk factors for schizophrenia (Ripke et al., 2014). It is now clear from these studies, however, that each of these common variants accounts for only a very small proportion of the overall heritability. It is estimated that common, low-risk variants contribute only 20-50% of the heritability for common, complex disorders (Wray, Goddard, & Visscher, 2008).

A second approach assumes that these conditions are caused by rare variants of larger effect size (Cirulli & Goldstein, 2010; Manolio et al., 2009). Copy number variants (CNVs) represent one example of such genetic variants (Mitchell, 2011; Sebat et al., 2007). CNVs are deletions, duplications, insertions, inversions, or complex recombinations of a small portion (50 base pairs to several megabases) of DNA. Since CNVs are rare events that often occur in isolated cases, they are not easily observable through GWA studies, which rely on detection of common variants that occur more frequently in cases than controls. CNVs are also too small to be detected through fluorescent in-situ hybridisation (FiSH), but can be observed through newer methods of microarray comparative in-situ hybridisation and exome sequencing (Slavotinek, 2008). Recent studies have indicated that rare copy number variations (CNVs) may contribute to the aetiology of ASDs and other neurodevelopmental disorders (Cook & Scherer, 2008; Grayton, Fernandes, Rujescu, & Collier, 2012; Mitchell, 2011). Indeed, CNVs occur more frequently in ASDs and in schizophrenia compared to the general population (Jacquemont et al., 2006; Levy et al., 2011; Sebat et al., 2007). These CNVs are most often de novo in origin, although inherited CNVs have also been observed in a smaller number of cases (Levy et al., 2011; Sebat et al., 2007; Xu et al., 2008).

CNVs may provide an easier "way in" to the study of the genetics underlying neurodevelopmental disorders. CNVs carry greater risk for disease than common variants, although penetrance is incomplete (Grayton et al., 2012; Vassos et al., 2010). In addition, although individual CNVs are rare, genome-wide analyses indicate that CNVs at particular chromosomal locations may confer particular risk for ASDs (Levy et al., 2011; Sanders et al., 2011). Finally, there is considerable convergence between the genes and pathways identified in SNP and CNV studies (Ripke et al., 2014).

Whilst attempts to uncover the genetics of complex neurodevelopmental disorders have identified risk variants across a wide range of genes, these risk variants appear to converge onto similar biological pathways. The majority of genetic risk variants identified in autism spectrum disorders involve genes that converge onto pathways involved in neuronal signalling and development, chromatin remodelling and synaptic function (Pinto et al., 2014). In particular, many risk factors for both schizophrenia and autism affect "synaptic" genes (Fromer et al., 2014; Kenny et al., 2014). This discovery converges with evidence from neuropathology and gene expression studies suggesting that impaired synaptic transmission contributes to schizophrenia and autism.

2.1.4 Mouse models of social cognitive impairment

One approach to exploring the contribution of genetic risk variants to neurodevelopmental disorders is through mouse models. Genetically modified mice can be used to investigate the circuits and mechanisms underlying different behaviours, including social behaviours.

Mice are a social species that engage in multiple forms of social interaction. Murine social behaviours include direct interaction, dominance and territory marking behaviours, aggressive behaviours and communal nest building. Although some aspects of social interaction are species-specific, mouse social behaviours are considered to bear some relevance to human social behaviours. Hence, mice can be used to explore the neural basis of simple social behaviours.

A number of tasks are available to study rodent social behaviours. Reciprocal social behaviours can be assessed in a direct social interaction task, which measures social behaviours such as sniffing and following, allogrooming and aggression. This provides a broad measure of the social behaviour displayed by the test mouse, and the ability to engage in reciprocal social behaviour. The motivation to engage in social behaviour (social

approach) can be more specifically assessed using a three-chamber social approach task, which measures the time a test mouse spends in a chamber containing a mouse placed under a wire cup compared to a chamber containing an object of similar size and shape (Nadler et al., 2004). By placing the social stimulus mouse under a wire cup, this provides a more stringent measure of sociability as it minimises the potential confound of the stimulus mouse's motivation to engage in, and initiating interaction. As a result, sociability of the test mouse can be easily quantified. This test is considered to model the abnormal social approach behaviours seen in neurodevelopmental conditions such as autism spectrum disorders. In addition, this task can be used to measure social recognition of conspecifics. This variant can be used to model social recognition deficits.

Other aspects of social behaviour that can be investigated in mice include aggressive behaviour, dominance and hierarchical behaviours, and social learning. Aggression can be investigated both during direct social interaction, and in a resident-intruder paradigm, which instigates territorial aggression in a "resident" mouse by placing an "intruder" mouse in the resident's homecage (Koolhaas et al., 2013). This task can be used to measure defensive and offensive aggression in a manner that is thought to bear resemblance to human aggressive behaviour. Social dominance has been studied using a "tube" test. Here, two mice are placed at opposite ends of a tube and dominance is assessed by identifying the mouse that first pushes the other out of the tube (Lindzey, Manosevitz, & Winston, 1966). Finally, social learning can be assessed using observational fear learning tasks. Observational fear learning tasks assess the ability of a rodent to learn to respond to an aversive stimulus through observation of a conspecific (Jeon & Shin, 2011). Some researchers have argued that this test may provide a test of empathy, although whether or not rodents possess empathy has been highly debated (Ben-Ami Bartal et al., 2011; Heyes, 1993; Silberberg et al., 2014). However,

as a minimum, this task provides a measure of the ability of mice to detect and use socially transmitted information.

Social communication can be measured in mice by examining olfactory communication and ultrasonic vocalisations. Mice emit ultrasonic vocalisations in a variety of situations, including upon separation from the nest/mother in pups, during play in juvenile animals, during male-female interactions, during aggressive encounters and in response to urinary deposits (Branchi, Santucci, & Alleva, 2001; Brudzynski, 2013; Nyby, 1983; Scattoni, Crawley, & Ricceri, 2009). These act as a form of communication, although their meaning is not well understood. In addition, olfactory signals appear to act as a communicative signal. Mice display high levels of interest in urinary scents, and can discriminate between different conspecifics from urinary deposits. Urinary deposits also act as a territorial marker. As a result, both ultrasonic vocalisations and scent marking behaviour are considered ethologically valid measures of communication deficits in neurodevelopmental disorders (Wöhr & Scattoni, 2013).

Nonetheless, there are limits to the study of social behaviours and modelling of social dysfunction in rodents. Whilst social motivation can be altered in individuals with impaired social cognition, alterations are also seen in the *quality* of social interaction. However, rodent tasks assessing social interaction are better able to measure the quantity of social interaction than the quality of social interaction. Tapping into the quality of rodent social interaction is difficult, because of our lack of understanding of the components of rodent social behaviour, and of what constitutes adaptive and non-adaptive interactions for rodents. In addition, multiple aspects of human social cognition appear beyond the scope of testing in mice. For example, there are no tests to measure emotion recognition, or knowledge of social "rules".

Some aspects of social cognition that depend on conscious awareness, such as theory of mind, or empathy, may not be displayed by mice.

2.1.5 The Neurexin genes

The mammalian neurexins are a set of three genes (NRXN1, NRXN2, NRXN3) encoding pre-synaptic cell-adhesion proteins. Each NRXN gene encodes two major isoforms (α-NRXNs and β-NRXNs) under the control of distinct promoters (Ushkaryov, Petrenko, Geppert, & Südhof, 1992). The larger α-NRXN mRNA is transcribed from the upstream promoter, generating a protein containing six extracellular N-terminal LNS (laminin, NRXN, sex-hormone-binding globulin) domains, whilst the shorter β-neurexin mRNA is transcribed from the downstream promoter, generating a protein with only one LNS domain. These genes possess multiple alternative splicing sites generating thousands of isoforms (Missler & Südhof, 1998; Ullrich, Ushkaryov, & Südhof, 1995). The large number of neurexin isoforms determines their binding partners and may contribute to specification of synaptic function (Aoto, Martinelli, Malenka, Tabuchi, & Südhof, 2013). Whilst neurexin genes were originally believed to play a role in synapse formation, more recent evidence has indicated a role in synaptic function (Born et al., 2015; Cao, 2017; Etherton et al., 2009; Missler, Zhang, Rohlmann, Kattenstroth, Hammer, Gottmann, & Südhof, 2003).

Mutations in neurexins have been linked with neurodevelopmental and psychiatric disorders. Microdeletions in the NRXN1 have been persistently associated with neurodevelopmental and psychiatric disorders. NRXN1 is located on chromosome 2p16.3 in humans and is one of the largest human genes (1.1 Mb with 24 exons). It encodes the neurexin-1 proteins, Neurexin-1α and neurexin-1β. Neurexin-1α was first discovered as a receptor for the neurotoxin alpha-latroxin (Geppert et al., 1998; Sugita, Khvochtev, & Südhof, 1999; Ushkaryov et al., 1992). It has since been found to play a role in synaptic function, in particular, in regulating pre-synaptic neurotransmitter release (Etherton et al., 2009).

Microdeletions in NRXN1-α display incomplete penetrance and can lead to a heterogeneous phenotype of varied severity (Todarello et al., 2014). The link between mutations in NRXN1 and neurodevelopmental/psychiatric conditions was first established for autism, where microdeletions in the promoter and first exon of NRXN1-α were identified as risk factors (Kim et al., 2008; Yan et al., 2008). Since then, mutations in NRXN1-α have also been found to predispose to a number of neurodevelopmental conditions, including schizophrenia, developmental delay, intellectual disability, language impairments, Tourette's syndrome and ADHD (Ching et al., 2010; Kirov et al., 2009; Rujescu et al., 2009; Schaaf et al., 2012; Shah et al., 2010; Sundaram, Huq, Wilson, & Chugani, 2010). Mutations in NRXN1-α are also associated with epilepsy (Schaaf et al., 2012). In addition, the homozygous deletion has been associated with a more severe Pitt-Hopkins like phenotype (Zweier et al., 2009).

2.1.6 Neurexin1-α

In humans, microdeletions in NRXN1-α cause impairments in social behaviour. In a case study of three families with family members possessing the NRXN1-α microdeletion, a common phenotype including dysexecutive behaviour including difficulties in working memory, attention switching, mental flexibility, and verbal fluency, impulsivity and heightened aggression was found across carriers (Viñas-Jornet et al., 2014).

The effect of the microdeletion has also been explored in mouse models carrying homozygous and heterozygous microdeletions in the murine neurexin-1-α gene (located on chromosome 17). An early characterisation of *Nrxn1α* knock-out mice maintained on a mixed SV129/C57 background identified only subtle behavioural changes, such as a deficit in nest building, altered pre-pulse inhibition, and increased grooming (Etherton et al., 2009). However, mutant mice maintained on mixed background strains are difficult to interpret as the background genetics, referred to as the congenic footprint, contributes to the behaviour and can confound interpretation of the phenotype (Gerlai, 1996; Schalkwyk et al., 2007). For

instance, the SV129 mouse line carries a mutation in Disc 1 (Koike, Arguello, Kvajo, Karayiorgou, & Gogos, 2006). In a full investigation of neurexin-1-α mice maintained on a pure C57BL6J background, knock-out mice were found to have profound alterations in social behaviour, characterised by increased aggression and altered social approach (Grayton et al., 2013). This displays *prime facie* similarity to the phenotype observed in human carriers of the heterozygote microdeletion (Viñas-Jornet et al., 2014).

Nrxn1α KO mice may constitute a unique model to study aspects of dysfunctional social behaviour that are distinct from social motivation. Indeed, these mice are one of the few mutant mouse models carrying a risk factor for neurodevelopmental disorders with a phenotype distinct from reduced social approach. Instead, *Nrxn1α* KO mice display increased aggression and heightened social approach. This contrasts with the majority of genetically modified mouse lines that display social impairments, where reduced social approach is observed (Born et al., 2015; Dachtler et al., 2014; Goorden, van Woerden, van der Weerd, Cheadle, & Elgersma, 2007; Katayama et al., 2016; Moy et al., 2009; Sato et al., 2012). Preliminary evidence suggests that this phenotype may reflect a deficit in recognising social cues and engaging in "appropriate" social behaviours. Indeed, *Nrxn1α* KO mice display aggression in contexts that do not typically elicit aggressive behaviours (e.g. in the presence of a juvenile mouse). In addition, a recent study investigating rats carrying the *Nrxn1α* homozygote deletion found that the rats displayed impaired social fear learning, indicating a deficit in using social signals to guide behaviour (Twining, Vantrease, Love, Padival, & Rosenkranz, 2017).

The phenotype of *Nrxn1α* KO mice is of interest for several reasons. First, these mice may model aspects of impaired social behaviour that are distinct from those displayed in other mouse models. Second, a full understanding of the neural basis of impaired social cognition

requires modelling aspects of social cognition that are separate from social motivation. Mice displaying reduced sociability are not always the best model to study other types of social impairments. A reduction in social motivation may confound studying other aspects of social behaviour, because mice with reduced sociability may show floor effects and therefore not display any social behaviour.

The aims of this study were to assess the validity of *Nrxn1α* KO mice as a model of social dysregulation in neurodevelopmental disorders. I aimed to replicate the original social phenotype reported in Grayton et al (2013), and to further explore this phenotype by investigating ultrasonic communication and response to olfactory social cues. Since specificity of impairment is also an important criterion for serving as a good model, a second aim was to determine whether the abnormal social behaviour seen in *Nrxn1α* KO mice is accompanied by a generalised cognitive impairment.

2.2 Methods

2.2.1 Animals and housing

Microdeletions in the human *Nrxn1α* or mouse *Nrxn1α* gene are technically not gene knockouts because the majority of the gene sequence remains. However, the *Nrxn1α* microdeletions that are risk factors for neurodevelopmental disorders commonly involve the promotor and first exon of the *Nrxn1α* gene. As a result, the effect of this microdeletion is similar to knocking out the entire *Nrxn1α* gene because expression of the neurexin-1α protein by the mutant gene is minimal. The mouse line that I am studying carries a microdeletion in the mouse *Nrxn1α* gene that involves the *Nrxn1α* promoter and the first exon. Hence, the mouse *Nrxn1α* microdeletion is similar to the human *Nrxn1α* microdeletion that is a risk factor for neurodevelopmental disorders. For simplicity, I will refer to mice carrying

homozygous *Nrxn1α* microdeletions as knock-outs (KO), while acknowledging that this is imprecise.

Male and female *Nrxn1α* KO mice were generated as described (Missler, Zhang, Rohlmann, Kattenstroth, Hammer, Gottmann, & Sudhof, 2003) and were genotyped by PCR using genomic mouse DNA from ear punches (WT primer: CGA GCC TCC CAA CAG CGG TGG CGG GA, KO primer: GAG CGC GCG CGG CGG AGT TGT TGA C, common primer: CTG ATG GTA CAG GGC AGT AGA GGA CCA). These mice had previously been maintained on a C57BL6/SV129 mixed genetic background (Etherton, Blaiss, Powell, & Südhof, 2009b). However, the SV129 strain contains background mutations which could create a possible confound (Koike et al 2006). To avoid this confound, *Nrxn1α* mice used in these experiments were backcrossed with C57BL/6J mice for 8 generations to establish a homogeneous background. Neurexin-1-alpha (*Nrxn1α*) WT (+/+), HET (+/-) and KO (-/-) mice used for behavioural and electrophysiological experiments were generated from *Nrxn1α* HET parents and genotyped as described previously (Hannah Mary Grayton et al., 2013).

Mice were bred and maintained in the Biological Services Unit (BSU) of the Institute of Psychiatry, Psychology & Neuroscience. Mice were housed in 32cm x 16cm x 14cm cages containing sawdust (Litaspen premium, Datesand Ltd, Manchester), bedding (Sizzlenest, Datesand Ltd, Manchester) and a cardboard shelter (Datesand Ltd, Manchester). Sawdust and bedding were replaced every other week but never on the day before or day of testing to minimise the potential effects of cage disturbance on the behaviour of the mice. Mice had *ad libitum* access to food (Rat and Mouse No. 1 and 3 Maintenance Diet for test and breeding mice respectively, Special Diet Services, Essex, UK) and water. Housing and testing rooms were carefully maintained at constant room temperature (21°C) and humidity (45%). Lights in the testing and housing rooms were maintained on a regular light/dark schedule with lights

on from 07:00 to 19:00 hours (light = 270 lux). Mice were tested between 08:00 am and 18:00 pm. Although testing rodents during the day may increase noise in behavioural data, previous experiments in our laboratory have indicated that day testing in low light conditions does not significantly alter behaviour in the selected behavioural tasks compared to a non-reverse light cycle.

The mice were weaned at 4 weeks of age. Animals were singly housed when adult to avoid potential confounds from social hierarchies and aggressive behaviour hierarchies, which could influence controlled assessment of social behaviours (Brown, 1953). For adult testing, mice were tested from 12 weeks of age onwards. Animals were tested in two batches (Batch 1: WT M = 7, WT F = 8, HET M = 12, HET F = 12, KO M = 10, KO F = 6; Batch 2: WT M = 7, WT F = 8, HET M = 8, HET F = 7, KO M = 8, KO F = 8). This was to ensure a sufficient number of mice in each genotype group per sex were tested while also keeping the possible confounding batch effects to a minimum. In the first batch the testing order consisted of: open field, novel object test, puzzle box, 3-chamber social approach task. In the second batch mice were tested in the following order: open field, novel object test, 3-chamber social approach task, Y maze spatial novelty task, Morris Water Maze. In addition, a third batch of male KO and WT mice underwent testing to assess ultrasonic vocalisations during male-female interactions (WT M = 10, KO M = 10). The oestrous phase of the female mice was not checked in this study, but it is unlikely that this affected the results as there were no major differences in the variance observed in the behavioural measures between males and females. C57BL/6J conspecific mice for the three-chamber task were obtained at 8 weeks of age from Charles River Laboratories (Margate, Kent, UK) and caged in 32cm x 16cm x 14cm cages containing sawdust (Litaspen premium, Datesand Ltd, Manchester), bedding (Sizzlenest, Datesand Ltd, Manchester) and a cardboard shelter (Datesand Ltd, Manchester). C57BL/6J mice were group-housed in groups of two, three, or four according to sex.

All tests were recorded using a camera positioned above the test arenas and movement of each mouse tracked using EthoVision software (Noldus Information Technologies, Wageningen, The Netherlands; http://www.noldus.com/site/doc200403002). After each trial, boli and urine were removed from the test arena which was then cleaned with 1 % Trigene® solution. At the end of testing, mice were returned to their home cage which was returned to the housing room.

Procedures and housing complied with the local ethical review panel of King's College London and the U.K. Home Office Animals Scientific Procedures Act 1986. All behavioural testing was conducted under licence (PPL: 70/7184; PIL: i28ea4c7f) and all efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2.2 Materials and procedures

2.2.2.1 Open Field

Materials

Based on spontaneous exploration of a novel environment, the open field is a well-validated anxiety test, first developed in 1951 (Hall & Whiteman, 1951), and it is one of the most widely used behavioral tests. It is based on the approach/avoidance conflict between the animal's tendencies to explore a novel area, and its avoidance of the aversive nature of the environment (openness), and does not require the use of strongly aversive stimuli or conditioning. Mice tend to avoid the central zone of the arena, staying in close contact with the walls of the open field and avoidance of the central zone has been used as a measure of anxiety induced by exposure to a novel environment. This wall-seeking or thigmotaxic behavior has been observed in several rodent species (Candland & Nagy, 1969) and is thought to reflect the propensity of small rodents to avoid open, unknown and potentially dangerous areas. The testing arena consisted of a 40 cm length x 40 cm width x 40 cm height

white square box containing enough sawdust to cover the entire base of the arena. The test was performed in the dark, with two red lights (LED cluster red light No. 310-6757; RS Components Northants, UK) with a wavelength of approximately 705nm) providing light for camera recording.

Procedure

Mice were placed in the testing arena for 10 minutes. The time spent in the inner and outer centre, and outer portions of the arena was measured using Ethovision software. In addition, the velocity (cm/s) and distance moved (cm) were recorded.

2.2.2.2 The novel object texture discrimination task

Materials

The novel object discrimination task takes advantage of the innate attraction of mice to novel objects (Misslin & Ropartz, 1981; van Gaalen & Steckler, 2000) to test memory of a familiar object in the presence of a novel object (Dere, Huston, & De Souza Silva, 2007). Short and long term memory may be assessed by presenting the novel object either 1 hr or 24 hrs after the first exposure to the familiar object, respectively. The time the mouse spends with the familiar versus the novel objects is taken as a measure of whether the animals can discriminate, and therefore have memory of, the familiar from the novel object.

Mice were tested in a 40 x 40 x 40 x cm testing arena (Fig. 1A-C). The objects used in the novel object discrimination task consisted of white rectangular plastic strips (4cm width x 15cm height) with a white circular base. Identical dark aluminium oxide sandpaper strips (4cm width x 7.5cm height) were attached on either side (Fig. 1A-C). Objects differed in terms of roughness but were visually unidentifiable. For habituation sessions, the arena was divided into an inner "centre" and outer square on Ethovision software to enable assessment

of the movement of the mice into the middle of the arena. The test was performed in the dark, with two red lights (LED cluster red light No. 310-6757; RS Components Northants, UK) with a wavelength of approximately 705nm) providing light for camera recording. The protocol was based on the study by Wu and co-workers (Wu, Ioffe, Iverson, Boon, & Dyck, 2013).

Procedure

Mice were first habituated to the testing arena in the absence of any objects. Mice underwent four 10-minute habituation sessions across four days, the first session of which consisted of the open field testing. Novel object preference was tested on the fifth day with two 10-minute trials (Fig. 1A-C). On the first trial, two identical objects were placed in the middle left and right of the testing box and the mouse was given the opportunity to explore the two objects. At the end of the trial the mouse was returned to its home cage for a 10 minute inter-trial interval. T0 ensure that the mice had explored the objects sufficiently in trial 1 to display novelty preference in trial 2, mice were required to investigate the objects for a minimum of 20 seconds across trial 1. Trial 2 began at the end of the inter-trial interval. In trial 2, one of the two identical objects was replaced with a different object possessing a different texture. The identity and location of the novel and familiar object was counterbalanced across genotype and sex. The time spent near each object was scored manually by an experimenter blind to genotype. In addition, distance moved (cm), velocity (cm/s), and time spent in the outer and inner portions of the arena were measured using Ethovision Software. The total time spent exploring objects was calculated on trials 1 and 2. In addition, novelty preference was calculated on trial 2 by computing a novelty discrimination ratio as follows:

[t(novel)/(t(novel) + t(familiar)]*100

where t(novel) is the time spent exploring the novel object and t(familiar) is the time spent exploring the familiar object.

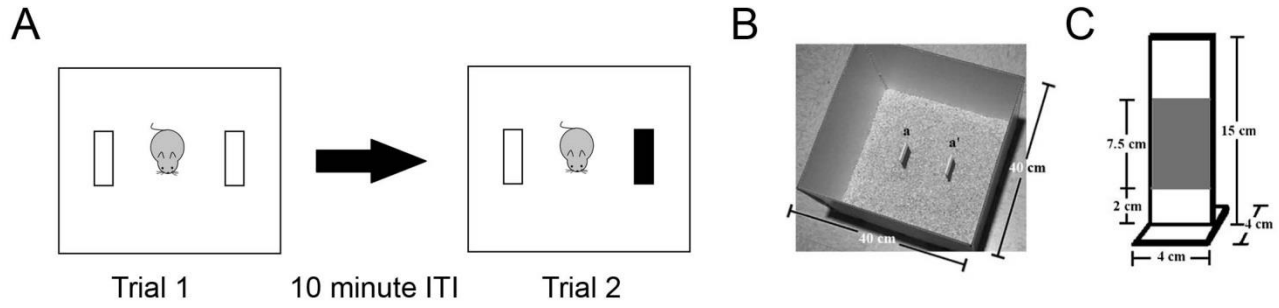


Figure 1: Procedure and materials used in the novel object texture discrimination task.

A: Diagram displaying procedure on the test day. Following a 10-minute trial with two identical objects, the test mouse is removed from the test apparatus for 10 minutes. This is followed by a 10-minute trial in which one object is replaced by a novel object. B, C: Photo of the testing chamber (B) and image of the objects (C), taken from Wu *et al.*, 2013.

2.2.2.3 Puzzle Box

Materials

The Puzzle box was introduced as a problem solving paradigm in previous studies on general cognitive ability in rats (Crinella & Yu, 1995). It consists of a series of problem solving tasks presented in order of difficulty. The puzzle box relies on the tendency of mice to avoid brightly illuminated open spaces as the motivation to solve the problems. A white plexiglass box (73cm length x 28cm x 27.5cm) was used for testing. The box was divided by a black plexiglass barrier into a smaller and a larger compartment. The smaller compartment contained sawdust and bedding, and was kept covered from light using a black plexiglass lid. The larger compartment was brightly lit with a lamp positioned to supply even illumination (100 lux). The two compartments were connected via a small underpass. One barrier dividing

the two compartments had a small open “door” above the underpass, while the other was a complete barrier (Fig. 2A). A small cardboard “plug” was used in some trials to block the underpass.

Procedure

Mice were trained to escape a brightly lit, aversive “start” box to reach a dark enclosed “goal” box. On each day, mice had to solve a new puzzle that blocked access to the “goal” box. The puzzles increased in difficulty across days (Fig. 2B). Mice were tested across five days on four different puzzles and underwent four trials per day, except on day five where there was only one trial. The first trial of each day consisted of a memory trial for the puzzle of the previous day, except on the first day; on the first trial of day 1, the “goal” box was easily accessible to the mice by means of a door in the barrier separating the “start” and “goal” compartments. This enabled mice to learn that the “goal” box was non-aversive. The next three trials on days 1-4 consisted of training on the new puzzle. On day five, there was only a memory trial. The puzzle on day 1 consisted of the “underpass” trial. Here, the door to the “goal” box was replaced by a complete barrier and mice had to learn to go through the underpass to reach the “goal” section. On day 2 (“sawdust 1”), the underpass was covered with sawdust and mice had to dig through the underpass to reach the “goal” section. On day 3 (“sawdust 2”), sawdust was placed over the underpass and surrounding top third of the “start” section. Mice had to locate the underpass and dig through sawdust to reach the “goal” section. For the last puzzle on day 4 (“plug”), a cardboard “plug” was placed in the underpass. Mice had to remove the plug to access the ‘goal section’. Mice completed 3 trials per puzzle for all trials expect the plug, for which they completed 4 trials. At the start of each trial, mice were placed in the “start” box facing away from the “goal” box and near the wall. Trials began when the mice were placed in the box and ended once all four paws had reached

the “goal” section, or after 3 minutes. The latency (s) to reach the “goal” section was recorded with a stopwatch by an experimenter blind to genotype.

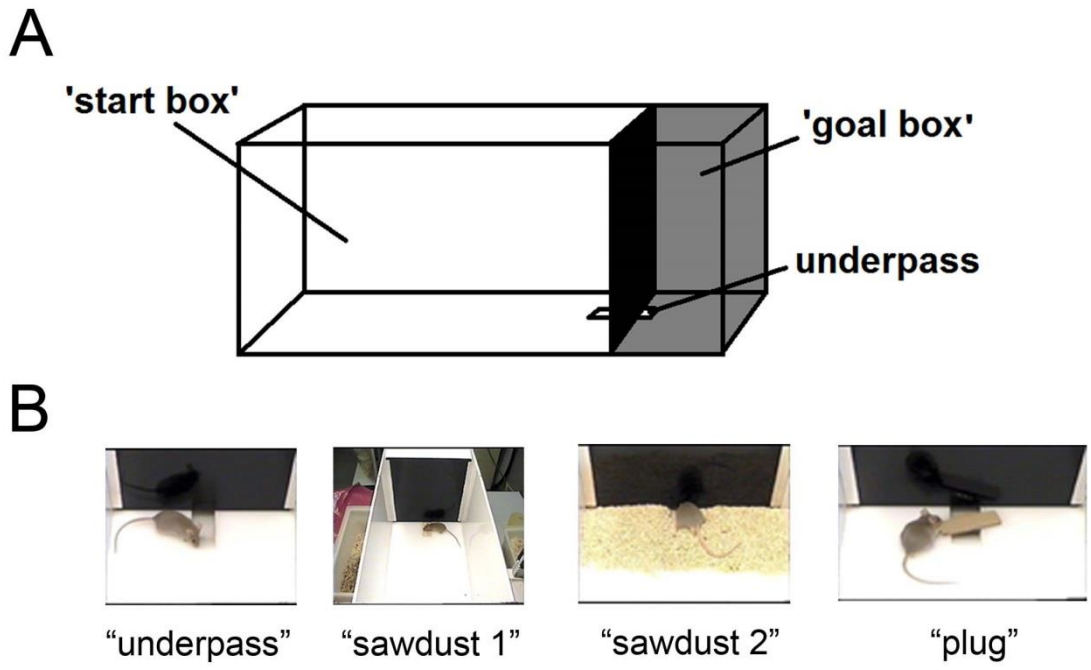


Figure 2: Puzzle Box apparatus and problem-solving tasks.

A: Diagram depicting the puzzle box apparatus. B: Photos of the four problem-solving tasks undergone by the test mice.

2.2.2.4 3-chamber social approach task

Materials

The three-chambered social approach task (modified from Yang *et al.*, 2011) monitors direct social approach behaviours when a subject mouse is presented with the choice of spending time with either a novel mouse or novel object. Sociability is defined as the subject mouse spending more time in the chamber containing the mouse than in the chamber containing the object. A subsequent 10-min trial is performed to measure short term memory. In this trial, the subject mouse is presented with the choice of either the familiar mouse from the previous

trial, or a novel mouse, after a 20 min inter-trial interval. Preference for the novel mouse is defined as spending more time in the chamber with the novel mouse than in the chamber containing the familiar mouse. The testing apparatus consisted of a transparent, rectangular, plexiglass box (60cm length x 40.5cm width x 22xm height) divided into three equal sized chambers (20cm length x 40cm width x 22cm height) by clear Perspex dividing walls (Fig. 3A-C). Access to the three chambers was provided by small openings in the centre of the dividing walls (5cm length x 8cm height). The testing box was covered with enough sawdust to cover the entire base of the arena. To prevent test mice from climbing on top of the wire cups, the testing arena was covered with a clear plexiglass lid. The wire cups under which the conspecific mice and novel objects were placed was 3.8-cm bottom diameter which ensures that the subject is close to the novel mouse when it moves around the cup. The gaps between the bars of the wire cup are large enough to permit visual, olfactory, acoustic and some tactile stimuli but small enough to prevent direct physical interactions. Clear plastic cups with lead weights were placed on top of the wire cups to keep them steady. The testing apparatus was placed on a trolley in the centre of the room. A small lamp positioned below the testing box provided even illumination (10 lux). The novel object consisted of a small black pointer of similar shape and size to the conspecific mouse.

Procedure

Mice were first habituated to the apparatus in the absence of any objects of conspecific mice for a 10 minute trial. Following this trial, novel object and novel conspecific mouse were placed under a wire cup in the top middle of one of the two side chambers, and an object was placed under a wire cup in the top middle of the other side chamber. The position of the mouse and object was counterbalanced across genotype and sex. The test mouse was able to explore the three chambers for a 10 minute trial. At the end of this trial, all mice and objects

were removed for a 20 minute inter-trial interval. In trial 3, the familiar conspecific mouse was placed back under the wire cup in the same chamber as before, whilst the novel object was replaced with a novel conspecific mouse. The test mouse was able to explore then chamber for 10 minutes (Fig. 3A-C). Conspecific mice were habituated to the wire cup for 10 minutes prior to trials 2 and 3 to avoid them displaying anxiety.

Mouse movements were tracked using Ethovision software. The time (s) spent in each chamber was recorded. In trial 2, the preference for the social chamber over the chamber containing the object was calculated using the formula:

[time(social)/(time(social) + time(object)]*100

In trial 3, the preference for the chamber containing the novel mouse over the chamber containing the familiar mouse was calculated using the formula:

[time(novel)/(time(novel) + time(familiar)]*100

In addition, the velocity of movement (cm/s) and distance travelled (cm) was recorded.

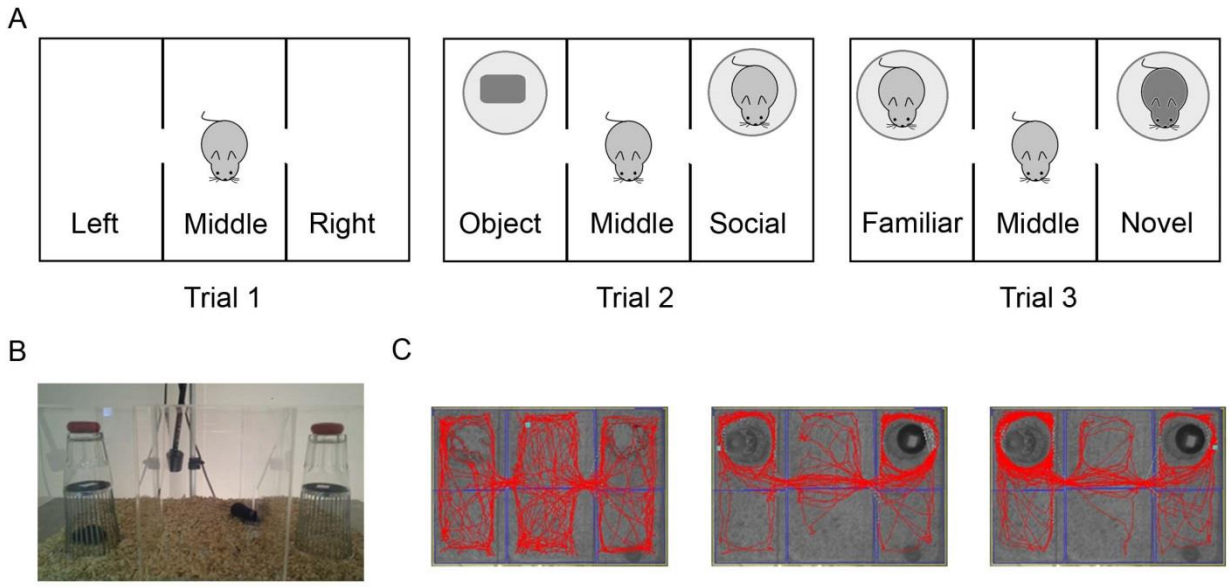


Figure 3: Procedure and materials used in the three-chamber social approach task.

A: Diagram depicting test procedure across the three trials. B: Image of the test apparatus. C: Example tracks across the three trials.

2.2.2.5 Ultrasonic vocalisations during male-female interaction

Materials

This task assesses the ultrasonic vocalisations made by adult male mice in response to interaction with an adult female mouse. This interaction is rewarding to male mice, and elicits 50-kHz "reward" ultrasonic vocalisations. As such, the task can be used to assess both the ability of a test mouse to communicate with other conspecifics, and to assess the affective valence of social interaction. The task was adapted from Scattoni, Ricceri and Crawley, 2011. Mice were tested in a standard homecage containing only sawdust. An ultrasonic microphone (Avisoft UltraSoundGate condenser microphone capsule CM16, Avisoft Bioacoustics, Berlin, Germany) was suspended 20 cm above the bottom, and 10 cm above the lid in the centre of the homecage. The microphone was sensitive to frequencies between 10 to 180 kHZ. The microphone was connected to a data acquisition device (Avisoft UltraSound Gate 116 Hm)

which connected via USB directly into a laptop. Vocalisations were recorded using Avisoft Recorder Software version 4.2. A sampling rate of 250 kHz, format 16 bit was used. Avisoft SASLab Pro (Version 5.2) was used for acoustical analysis. A fast fourier transform was performed to generate spectrograms with an FFT-length of 1024 points and a time window of 75% (100% Frame, Hamming window). Frequencies below 20 kHz were removed with a cut-off to remove background noise.

The mice used as conspecific female mice consisted of 10 littermate *Nrxn1α* heterozygous mice.

Procedures

Mice were habituated to the fresh homecage for 1 hour prior to testing. Upon being brought to the test room, test mice were habituated to the room in the test homecage for 10 minutes. At the end of the habituation period, a female mouse was introduced into the homecage. Ultrasonic vocalisations made by the male mice were captured for a 2 minute trial.

2.2.2.6 Y Maze Spontaneous Spatial Novelty Discrimination

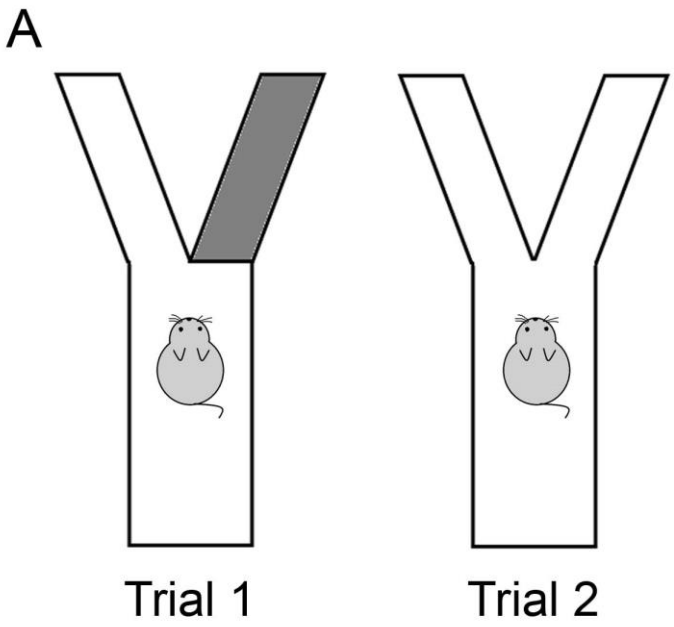
Materials

This task takes advantage of the innate attraction of mice to novelty to test memory of a familiar location after exposure to a novel location (Bannerman et al., 2008). Short and long term memory may be assessed by presenting the novel location either 1 hr or 24 hrs after the first exposure to the familiar location, respectively. This test assesses rapidly acquired, short-term (STM) and long-term (LTM) spatial memory and relies on the fact that normal mice prefer novel over familiar spatial environments. A subsequent trial (trial 4) is run a few days later with all three arms (plus start arm) open to test for arm preference. Mice were tested in a transparent plexiglass 8-arm radial maze in which five arms were blocked off to form a Y

Maze (Fig. 4A, B). This made a maze with three arms which were 22 cm long, 7 cm wide, with 20-cm-high walls. Black rectangular plexiglass sheets were used as doors to block off the Y maze arms. Extra-maze cues were placed on the curtains and walls surrounding the maze. Mice were assigned two arms ("start arm" and "other arm"), to which they were exposed during the first trial of the test (the exposure phase). During this exposure phase, the entrance to the third arm of the maze (the "novel arm" for the subsequent trial 2 was blocked off with a sheet of opaque Perspex. For trial 2, familiar, novel and start arms were open. Cues are placed outside the maze to aid spatial navigation. The testing area was kept in low light conditions (10 lux).

Procedure

Mice underwent three 5 minute trials. On trial 1, access to either the left or right "Y" arms was blocked with a dark plexiglass "door". The identity of the blocked arm was counterbalanced across genotype and sex. At the end of trial 1, mice were removed from the apparatus and returned to their homecage and housing room. Mice underwent trial 2 one hour after the start of trial 1. In this trial, the door was removed to allow access to both the left and right arms of the Y maze (Fig. 4A, B). One week later, the mice underwent a third trial to check for side preference. In trial 3, both arms again remained open. Before each trial, mice were placed in the "start" arm facing the wall. The start of each trial began when the mouse exited the "start" arm for the first time. An entry into and out of an arm was defined as all four paws of the mouse entering or exiting the arm. Mouse movements were tracked using Ethovision software. The time spent in each arm and frequency of entries into each arm was measured. In addition, velocity (cm/s) and distance travelled (cm) were tracked.



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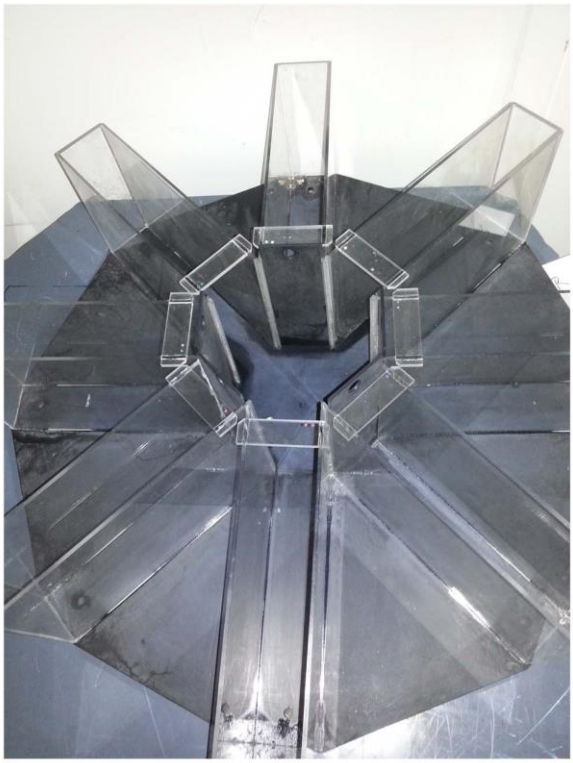

Figure 4: Procedure and materials used in the Y maze spontaneous novelty preference task.

A: Diagram depicting procedure on trials 1 and 2. B: Image of test materials.

2.2.2.7 Morris Water Maze

Materials

This is a standard and commonly used spatial navigation task that requires mice to swim to a submerged platform (target) by means of triangulation using navigational cues placed outside the pool (Morris, 1984). Mice were tested in a grey circular pool filled with tap water up to 30 cm depth (Fig. 5A). Since *Nrxn1α* KO mice were previously reported to show a reduced swim speed compared to HET and WT mice in a larger (1.3 m diameter, 60 cm high) pool (Grayton et al., 2013), a smaller pool was used in these experiments. To prevent mice from visually locating the platform, the water was made opaque using a non-toxic, white aqueous emulsion (Acusol OP301 Opacifier, Rohm & Haas, Landskrona, Sweden). Extra maze cues were hung from the curtains and wall surrounding the pool to aid spatial navigation. The platform was 29 cm high and had a round surface (10 cm diameter). The platform was positioned just above the water level on day 1 and 1 cm below water level of subsequent days. The pool was divided into four equidistant quadrants designated Target (T), Opposite (O), Left (L) and Right (R). In addition, the pool was divided into an inner and outer ring. Faecal boli were removed between trials and water in the pool was changed weekly.

Procedure

Mice were taken to the testing room in squads of six. Each session consisted of four trials per mouse and mice underwent four trials per day (i.e. one session a day). Mice were tested in squads of six and a new trial started when all 6 mice had finished the previous trials. Mice were returned to their home cages between trials and were returned to their housing room at the end of the session once all mice in the squad had been tested. Males and females were run in separate groups. Trials began by placing the mouse in the pool close to, and facing, the pool wall in one of the four start quadrants (Target, Opposite, Left and Right). The order of

testing starting in each quadrant was pseudorandom and was designed to ensure that mice could not rely on a fixed route to reach the platform (Fig. 5B). On each day, mice underwent one trial starting from each start quadrant. On the first day, the platform was visible and mice were trained to swim to the visible platform. On the subsequent 6 days, the platform was hidden below water level and mice had to locate the platform from memory. Trials ended once the mouse reached and climbed onto the platform, or after 60 seconds if the mouse did not reach the platform. The trial started once the mouse was placed in the pool. If mice did not manage to locate the platform after 60 seconds, the experimenter carefully guided the mouse to the platform with a wooden plank. Mice were allowed to remain on the platform for 30 s before being removed by the experimenter. On day 8, the location of the platform was shifted to the Opposite quadrant to assess reversal learning. Reversal learning was tested across five days using the same procedure. On the last day of spatial learning (day 7) and the last day of reversal learning (day 12), a fifth "probe" trial lasting 60 seconds was conducted in which the platform was removed from the pool. To assess the retention of spatial memory, the time spent in the quadrant that had contained the platform (target quadrant) compared to the other quadrants was measured. The latency to reach the platform was measured by an experimenter blind to genotype using a stopwatch. In addition, the swim path length (cm), swim speed, and time (s) spent in each quadrant was measured using Ethovision software. Mean latencies (s) and path lengths (cm) were calculated across the trials within each session for each mouse. Conflicting behavioural responses such as floating or thigmotaxis (the amount of time spent swimming in the outer area of the pool defined as a 15 cm wide circular zone adjacent to the wall of the maze) were assessed throughout the trials.

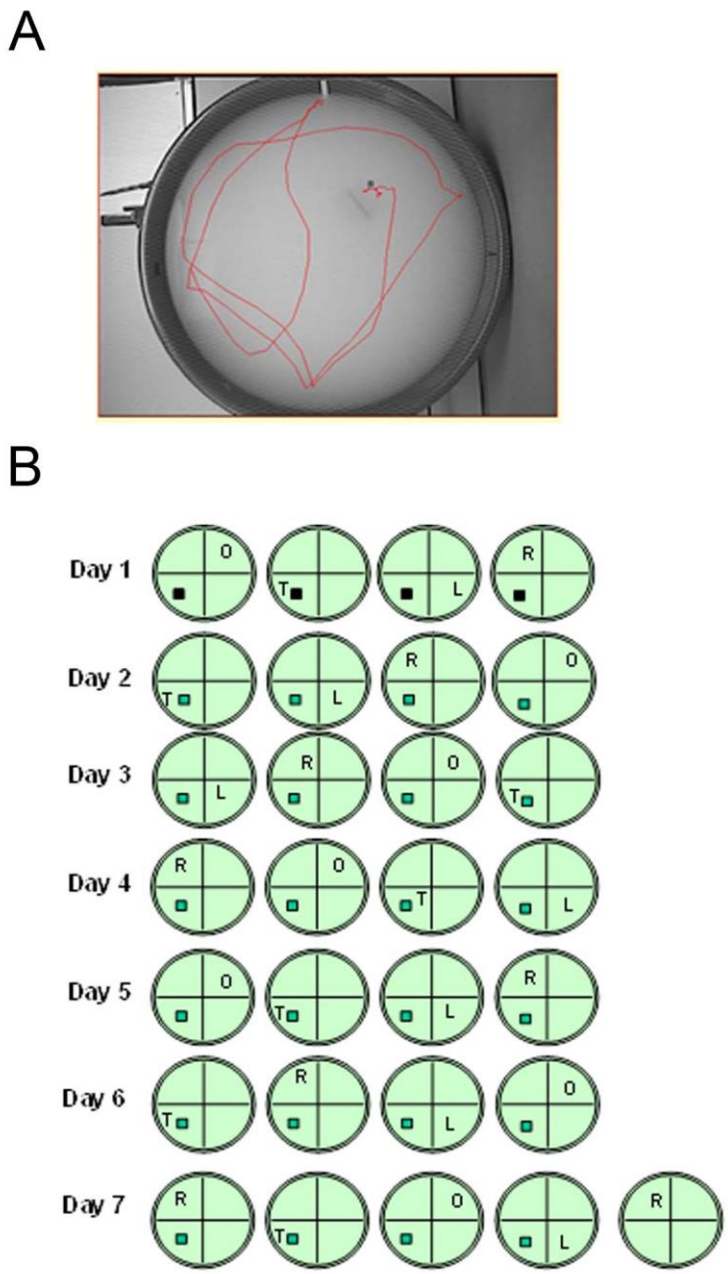

Figure 5: Materials and procedure in the Morris Water Maze task.

A: Image of testing apparatus and tracking. B: Diagram depicting the task procedure across test days. Mice underwent four trials a day starting from a different starting zone (indicated by the letter in the quadrant). On day 7, mice underwent a fifth probe trial.

2.2.3 Statistical Analysis

Data were analysed using SPSS. Mean, standard deviation (s.d.) and standard error (s.e.m.) were computed. Data that were non-normally distributed or that violated the assumption of equal variance were log transformed. If the data were still non-normally distributed, the median and interquartile ranges were computed and the data were analysed with non-parametric statistics. As a result of the large number of conditions, the means and standard errors for the data in this chapter are reported as tables in Appendix 1 (Tables 8-24), rather than in the text.

Data were analysed with a between-subjects or a mixed analysis of variance (ANOVA) as appropriate. For anxiety tests, an ANCOVA was used with locomotor activity as a covariate to control for any effects of locomotor activity. Post-hoc Bonferroni corrected t-tests were conducted to explore any main and interaction effects further. In addition, one-sample t-tests comparing performance to chance (50%) were conducted in the Novel Object Discrimination and Y Maze Spontaneous Spatial Novelty tasks to assess novelty preference.

2.3 Results

2.3.1 *Nrxn1α* KO mice display heightened anxiety

I first examined whether *Nrxn1α* KO mice displayed changes in anxiety. Measures of anxiety are important to conduct in the context of cognitive assessments of genetically modified mice because heightened anxiety is a possible confounds in the interpretation of other tasks; mice with high levels of anxiety can appear impaired in cognitive due to reduced levels of exploration. In addition, neurodevelopmental disorders often present with comorbid anxiety (Simonoff et al 2008; van Steensel et al 2011).

Anxiety was assessed using an open field arena (Hall & Ballachey 1932; Hall 1934). *Nrxn1α* KO mice displayed a number of changes in exploration of the open arena. Male and female

Nrxn1α KO mice made significantly fewer entries into the centre of the arena (Genotype factor: $F(2, 95) = 33.201$, $\eta^2 = 0.411$, $p < 0.001$; post-hoc t-tests: KO vs WT: $p < 0.001$; KO vs HET: $P < 0.001$, Fig. 6A, B). In addition, female but not male KO mice spent significantly less time in the centre of the arena (Genotype factor: $F(2, 95) = 20.253$, $p < 0.001$; Genotype x Sex factor: $F(2, 95) = 3.835$, $p = 0.025$, post-hoc t-tests: KO F vs WT F: $p < 0.001$, HET F vs KO F: $p < 0.001$, female KO vs male KO: $p = 0.043$, male KO vs male HET: $p = .024$, Fig. 6C, D). Male and female *Nrxn1α* KO mice also travelled a significantly shorter distance in the arena compared to HET and WT mice ($F(2, 95) = 6.958$, $p = 0.002$; post-hoc t-tests: KO vs WT: $p = 0.004$; KO vs HET: $p = 0.005$, Fig. 6E, F). These effects remained significant after controlling for any differences in locomotor activity, indicating that the changes in exploration were not secondary to differences in locomotor activity. Overall, the reduced exploration of the anxiogenic centre of the arena indicates that *Nrxn1α* KO mice display increased anxiety relative to HET and WT mice.

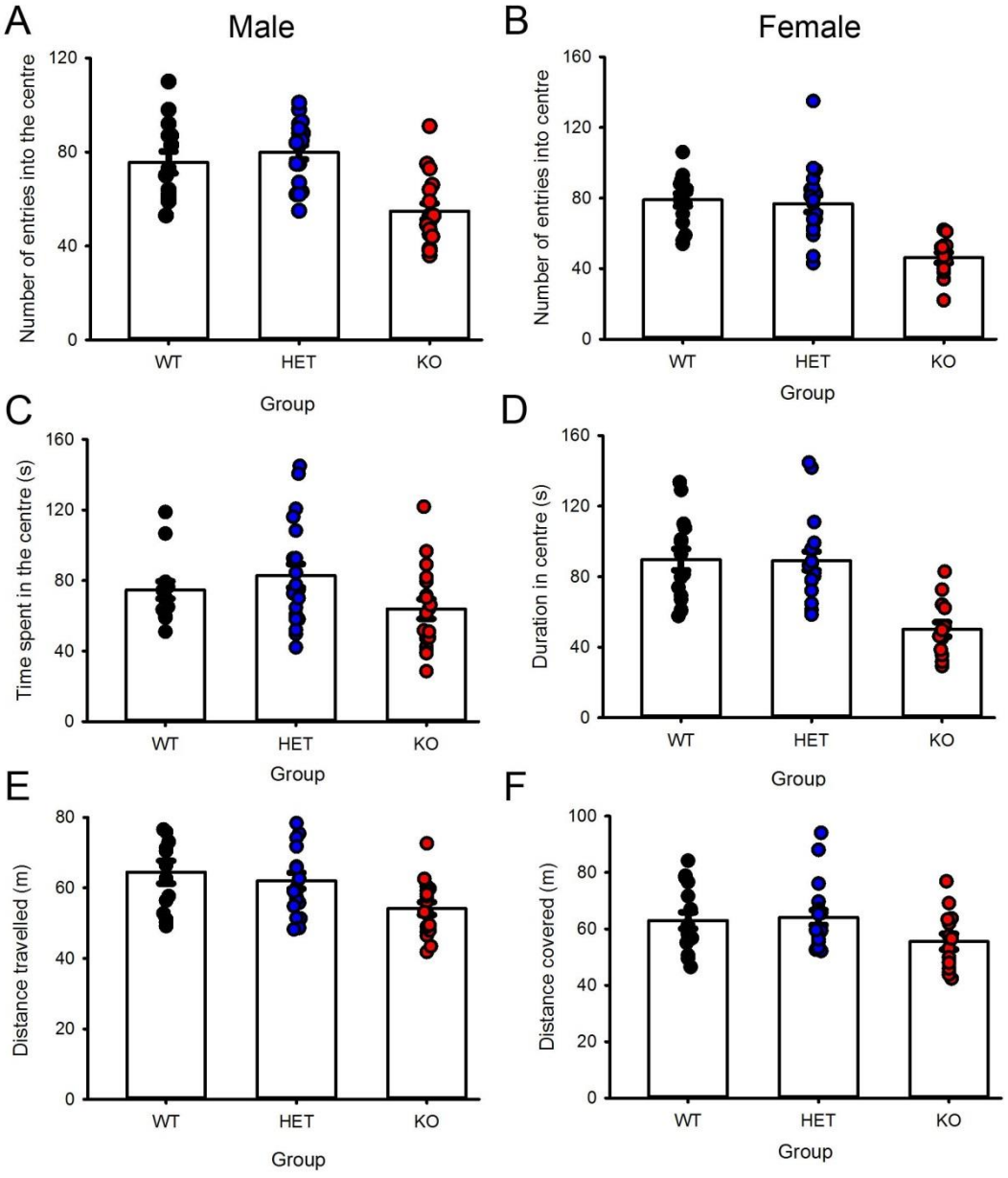

Figure 6: Increased anxiety and reduced exploration in *Nrxn1α* KO mice.

A, B: Mean ± standard error (sem) number of entries into the central portion of the arena in male (A) and female (B) mice with overlaid scatter. C, D: Mean ± sem time spent in the central portion of the arena in male (C) and female (D) mice with overlaid scatter. E, F: Mean ± sem distance travelled in the arena in male (E) and female (F) mice with overlaid scatter. Number of mice: WT M = 14, WT F = 16, HET M = 20, HET F = 19, KO M = 18, KO F = 14.

2.3.2 *Nrxn1α* KO mice show abnormal social behaviour

Abnormal social behaviour is one of the defining symptoms of ASDs (DSM-V), and is a feature of other neurodevelopmental disorders such as schizophrenia and William's syndrome. Many genetically modified mouse models carrying genetic risk factors for ASDs display reduced social approach, suggesting reduced social motivation. In contrast, *Nrxn1α* KO mice have previously been found to display abnormally heightened social approach (Grayton et al 2013). I sought to replicate and extend this finding to further explore the nature of abnormal social behaviour in *Nrxn1α* KO mice.

2.3.2.1 Three-chambered social approach task

Social approach behaviours were examined using the three-chamber apparatus. *Nrxn1α* KO mice travelled a significantly shorter distance in the 10-minute habituation trial (Genotype factor: $F(2, 95) = 18.796$, $\eta^2 = 0.284$, $p < 0.001$, post-hoc tests: KO vs WT: $p < 0.001$, KO vs HET: $p < 0.001$, Fig. 7). This mirrored the reduced exploration observed in *Nrxn1α* KO mice in the open field task.

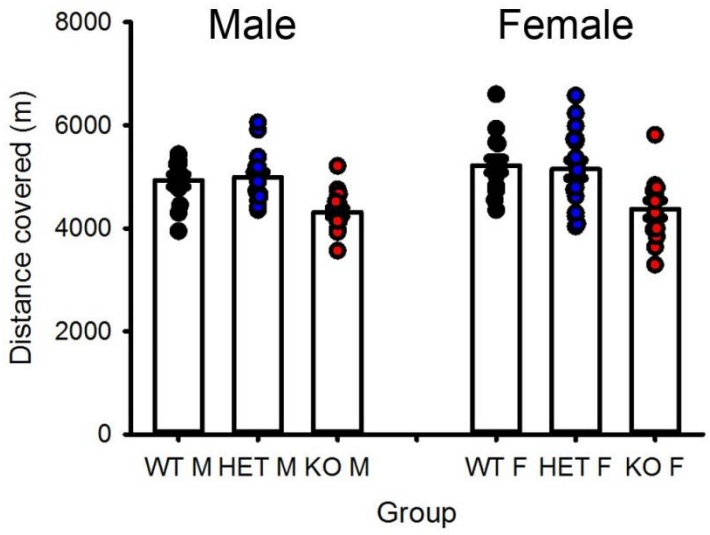

Figure 7: Exploration of 3-chamber apparatus during habituation.

Data are mean ± standard error (sem) distance covered in the 3-chamber apparatus across trial 1 in male and female *Nrxn1α* WT, HET and KO mice. Numbers of mice: WT M = 14, WT F = 16, HET M = 20, HET F = 19, KO M = 18, KO F = 14.

Trial 2 assessed the preference of mice for a novel conspecific mouse over a novel object by measuring the time spent in the chamber containing the novel object and in the chamber containing the social stimulus. One-sample t-tests comparing percentage preference for the social stimulus to chance were significant across all groups (WT M: $p = 0.003$, WT F: $p < 0.001$, HET M: $p = 0.001$, HET F: $p < 0.001$, KO M: $p < 0.001$, KO F: $p < 0.001$). All mice spent significantly longer in the chamber containing the conspecific mouse than the chamber containing the object (stimulus type factor: $F(1,95) = 283.111$, $\eta^2 = 0.749$, $p < 0.001$). However, *Nrxn1α* KO mice spent significantly longer in the chamber containing the conspecific mouse than did other mice (genotype x stimulus interaction: $F(2,95) = 6.761$, $\eta^2 = 0.125$, $p = 0.002$, post-hoc t-tests comparing genotype within social chamber: WT vs KO: p =

0.001, KO vs HET: p = 0.003, Fig. 8A). In addition, female mice spent significantly longer in the chamber containing the conspecific mouse than male mice (stimulus x sex interaction: $F(1,95) = 7.950$, $\eta^2 = 0.077$, $p = 0.006$). Similar results were obtained when comparing percentage preference for the social chamber over the novel chamber. *Nrxn1α* KO mice displayed significantly greater preference for the social chamber compared to WT and HET mice (genotype factor: $F(2,95) = 4.657$, $\eta^2 = 0.89$, $p = 0.012$, post-hoc t-tests: WT vs KO: $p = 0.025$, WT vs HET: $p = 0.031$, Fig. 8B, C). In addition, female mice displayed greater preference for the social chamber than male mice (sex factor: $F(1,95) = 8.706$, $\eta^2 = 0.084$, $p = 0.004$). Overall, these results indicated that *Nrxn1α* KO mice display heightened social approach.

In trial 3, the object was replaced with a novel conspecific mouse to test social memory. In this trial, preference for the novel mouse over the familiar mouse could indicate memory for the familiar mouse. One-sample t-tests comparing percentage novelty preference indicated significant novelty preference in the male WT and in all female mice, but not in male KO and HET mice (WT M: $p = 0.029$, WT F: $p < 0.001$, HET M: $p = 0.610$, HET F: $p = 0.001$, KO M: $p = 0.584$, KO F: $p = 0.002$). However, the time spent in the novel and familiar chambers was not significantly different across genotypes (genotype factor x chamber: $F(2,95) = 1.319$, $\eta^2 = 0.027$, $p = 0.272$, Fig. 8D). Female mice spent significantly longer in the chamber containing the novel mouse than did male mice (sex x chamber interaction: $F(1, 95) = 17.825$, $\eta^2 = 0.158$, $p < 0.001$). In addition, *Nrxn1α* KO male and female mice spent significantly longer exploring both conspecific mice than the HET and WT mice, supporting the heightened social approach observed in trial 2 (Genotype factor: $F(2, 95) = 5.051$, $\eta^2 = 0.096$, $p = 0.008$). Similar results were observed when measuring percentage novelty preference. Percentage novelty preference was not significantly different across genotype (genotype factor: $F(2,95) = 1.249$, $\eta^2 = 0.026$, $p = 0.291$, Fig. 8E, F). There was a significant

effect of sex, indicating greater % novelty preference in female mice compared to male mice ($F(1,95) = 18.442$, $\eta^2 = 0.163$, $p < 0.001$).

The absence of a significant interaction between genotype and chamber indicated that there were no significant differences in preference for a novel mouse after a delay across *Nrxn1α* KO, HET and WT female mice. Interpretation of this trial is somewhat limited in male mice. However, the results suggest that social novelty preference over a delay is intact in *Nrxn1α* HET and KO female mice.

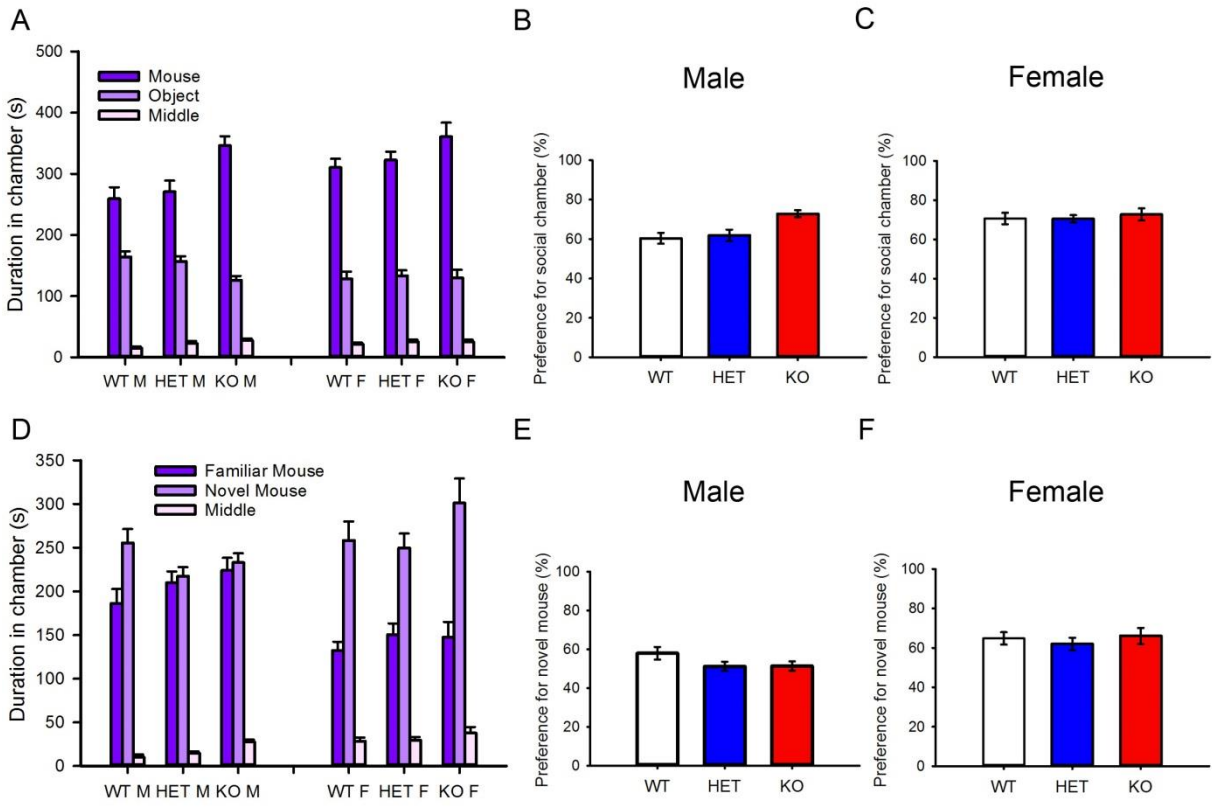

Figure 8: The three-chamber social approach task.

A: Mean ± sem time spent in the object, middle, and social chamber in *Nrxn1α* WT, HET and KO mice across trial 2. B, C: Mean ± sem preference for social chamber in male (B) and female (C) mice across trial 2. D: Mean ± sem time spent in the novel, middle, and familiar chamber in *Nrxn1α* WT, HET and KO mice across trial 3. E, F: Mean ± sem preference for chamber containing novel mouse in male (F) and female (G) mice across trial 3. Numbers of mice: WT M = 14, WT F = 16, HET M = 20, HET F = 19, KO M = 18, KO F = 14.

2.3.2.2 Adult ultrasonic vocalisations during male-female interactions

To further explore the socio-communicative phenotype of *Nrxn1α* KO mice, I investigated whether adult male *Nrxn1α* KO displayed altered ultrasonic vocalisations during male-female interactions.

There was no significant difference in the duration or frequency of ultrasonic vocalisations made by male *Nrxn1α* WT and KO mice (number: WT: 666 [537-711], n = 10 mice, KO: 593 [529-627], n = 10 mice, U = 38.50, p = 0.406, Mann-Whitney U test, Fig. 9A; duration: WT: 41 ± 6 ms, n = 10 mice, KO: 34 ± 6 ms, n = 10 mice, t(18) = 0.813, p = 0.427, Fig. 9B).

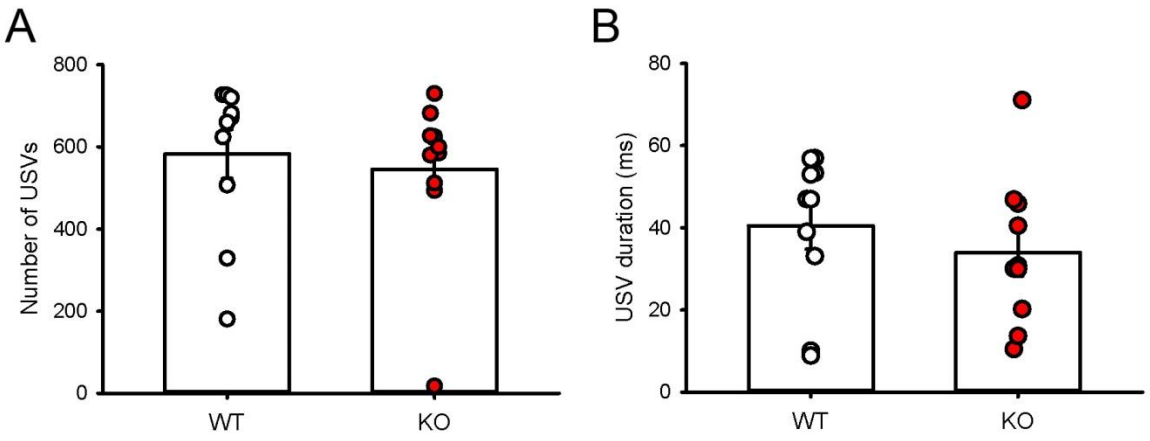

Figure 9: Ultrasonic vocalisations (USVs) in male *Nrxn1α* KO and WT mice.

A: Mean ± sem number of USVs with overlaid scatter in WT (empty circles) and KO (red circles) mice.

B: Mean ± sem duration of USVs with overlaid scatter in WT (empty circles) and KO (red circles) mice.

I concluded that ultrasonic vocalisations in response to interaction with females were unaltered in *Nrxn1α* KO mice.

2.3.3 *Nrxn1α* microdeletion does not affect short-term object memory

2.3.3.1 Habituation

Habituation is a simple form of learning in which an animal reduces responding to a stimulus with repeated presentations. Habituation to the novel object testing arena was assessed by measuring the distance travelled in the testing arena across multiple days. Reduced exploration of the arena across repeated exposure to the testing apparatus indicates habituation to the apparatus.

Male and female *Nrxn1α* KO mice travelled a significantly shorter distance on day 1 than HET and WT mice. Only HET and WT mice displayed reduced exploration across testing days (Day factor: $F(3,285) = 20.631$, $\eta^2 = 0.178$, $p < 0.001$; (Day x Genotype factor: ($F(6,285) = 5.631$, $\eta^2 = 0.106$, $p < 0.001$; KO vs WT: $p = 0.004$, KO vs HET: $p = 0.005$, Fig. 10A, B).

Overall, these results indicated that HET and WT mice habituated to the testing arena. KO mice failed to habituate to the testing arena. However, this effect is likely driven by their reduced exploration as a result of their greater anxiety on day one. Hence, there may have been a floor effect on the performance of *Nrxn1α* KO mice, as their levels of exploration on day one were already similar to the levels of exploration displayed by WT and HET mice on day four.

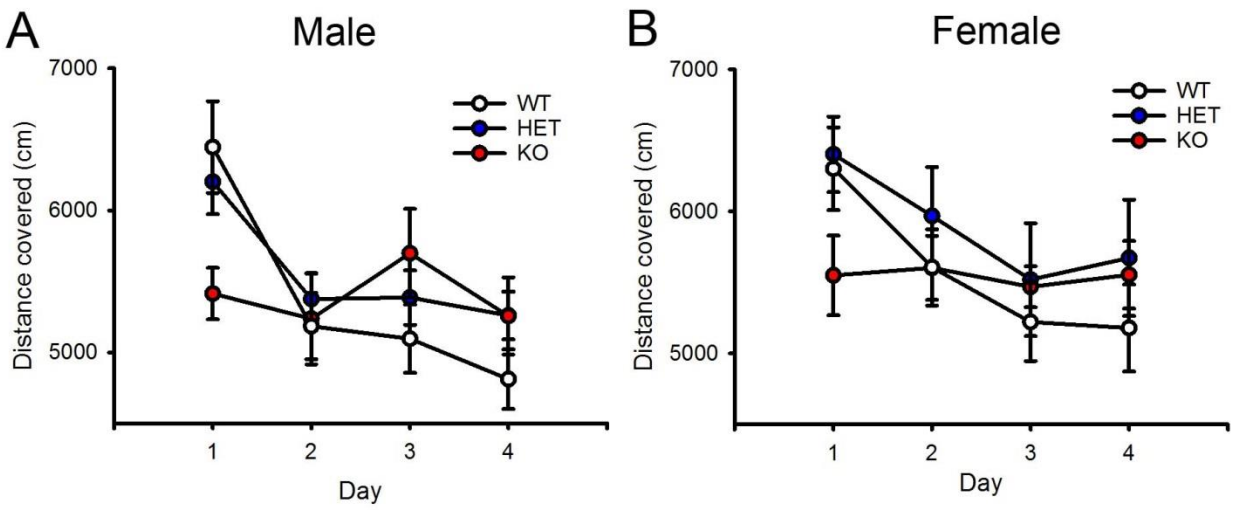

Figure 10: Habituation to the novel object testing arena.

Mean ± sem distance travelled in the testing arena across days in male (A) and female (B) *Nrxn1α* WT, HET and KO mice. Numbers of mice: WT M = 14, WT F = 16, HET M = 20, HET F = 19, KO M = 18, KO F = 14.

2.3.3.2 Short-term object memory

Next, I investigated short-term object memory through the novel object task. Total object exploration was measured on trial one, to ensure that all mice sufficiently explored the familiar objects. All mice explored the objects for more than 20 seconds, which was the minimum requirement for object discrimination. A significant genotype effect indicated that HET male and female mice spent significantly longer exploring both objects than KO mice, but not WT mice ($F(2,98) = 7.946$, $\eta^2 = 0.146$, $p = 0.001$, KO vs HET $p < 0.001$, WT vs HET $p = 0.063$, Fig. 11A). HET mice also made significantly more exploration bouts of the objects than KO and WT mice ($F(2,98) = 6.052$, $\eta^2 = 0.115$, $p < 0.001$; HET vs WT: $P = 0.044$, HET vs KO: $p = 0.004$, Fig. 11B). In addition, female mice explored the objects more frequently than male mice ($F(1,98) = 7.394$, $\eta^2 = 0.074$, $p = 0.008$). Hence, HET mice displayed a slightly elevated object exploration compared to WT and KO mice.

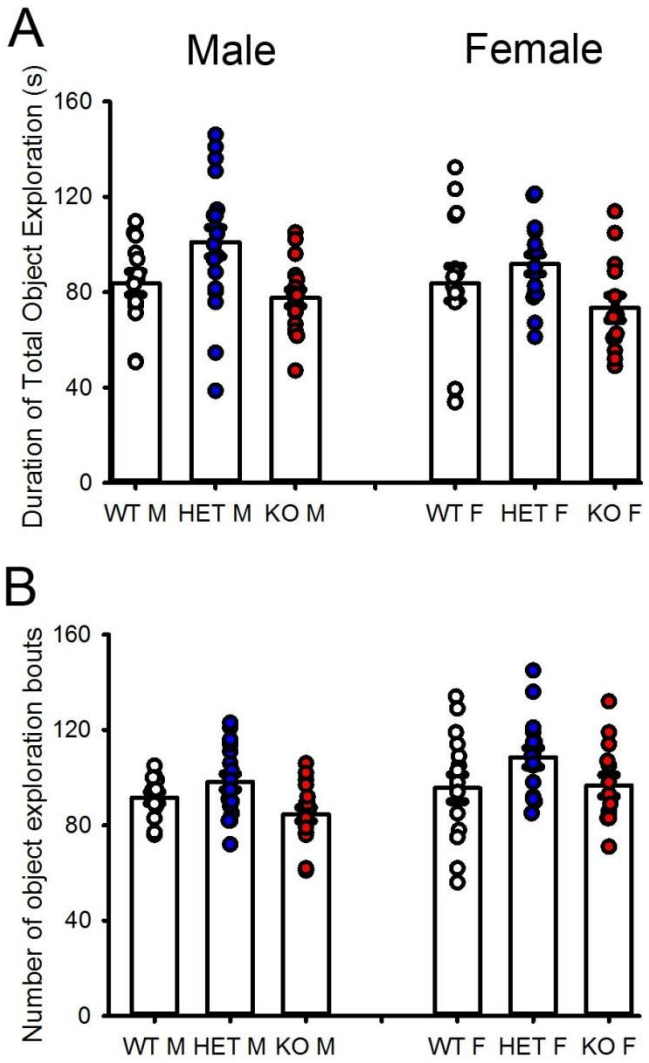

Figure 11: Trial 1 object exploration in *Nrxn1α* WT, HET and KO mice.

A: Mean ± sem object exploration duration with overlaid scatter in WT, HET and KO male and female mice. B: Mean ± sem number of object exploration bouts with overlaid scatter in WT, HET and KO male and female mice. Numbers of mice: WT M = 14, WT F = 16, HET M = 20, HET F = 17, KO M = 18, KO F = 14.

Preference for the novel object over the familiar object was measured in trial two. Novelty preference peaked 2 minutes into the trial and decreased across time in the trial (time factor: $F(2,186) = 13.296$, $\eta^2 = 0.125$, $p < 0.001$: 2 mins vs 5 mins: $p = 0.006$, 2 mins vs 10 mins: $p < 0.001$, 5 mins vs 10 mins: $p = 0.017$, Fig. 12A-C). One-sample t-tests comparing performance

to chance performance (50% novelty preference) were significant only for WT and HET males, but not KO males, WT, HET or KO females. However, novelty preference was not significantly different from chance in WT and HET males (p = 0.083). There were no significant effects of genotype or interaction effects (genotype factor: $F(2,93) = 0.476$, $\eta^2 = 0.010$, $p = 0.623$, genotype x sex factor: $F(2,93) = 2.667$, $\eta^2 = 0.054$, $p = 0.075$, time x genotype factor: $F(4,186) = 0.307$, $\eta^2 = 0.007$, $p = 0.799$, time x genotype x sex factor: $F(4,186) = 1.509$, $\eta^2 = 0.031$, $p = 0.219$).

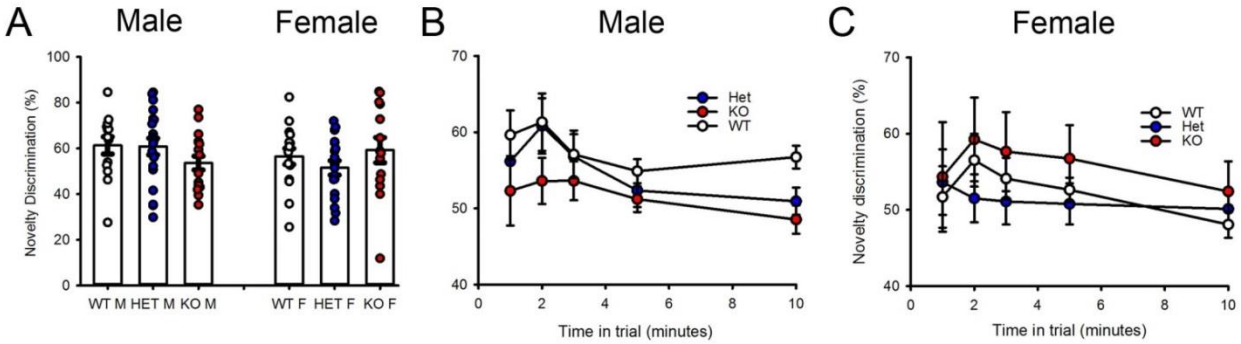

Figure 12: Novel object performance in *Nrxn1α* WT, HET and KO mice.

A: Mean ± sem novelty preference at two minutes in WT, HET and KO male and female mice, with overlaid scatter. B,C: Mean ± sem novelty preference across time in the test trial in male (B) and female (C) mice. Numbers of mice: WT M = 14, WT F = 16, HET M = 20, HET F = 18, KO M = 17, KO F = 14.

These results suggest that KO and HET mice do not perform differently from WT in short-term object memory. However, interpretation of the novel object discrimination task is limited by the weak novelty preference in WT mice. Given the limitations of the task, I sought to explore short-term memory in a spatial Y maze task.

2.3.4 Short-term and long-term spatial memory is intact in *Nrxn1α* KO and HET mice

2.3.4.1 Y Maze

Whilst they are not a characteristic feature of autism spectrum disorders, memory impairments are sometimes reported in individuals with ASDs and are observed in other neurodevelopmental disorders. In addition, autistic individuals often display altered spatial processing styles (Frith 1989; Frith & Happe 1994). Hence, spatial memory is an important domain to include in murine cognitive assessments.

Short-term spatial memory was assessed in a Y maze. Preference for the novel arm was significantly greater than chance in WT female, HET female and male, and KO male mice, but not WT male and KO female mice (WT M: $p = 0.375$, WT F: $p = 0.001$, HET M: $p = 0.009$, HET F: $p < 0.001$, KO M: $p = 0.041$, KO F: $p = 0.217$). There were no genotype differences in novelty discrimination in either the duration or frequency measures (duration: genotype factor: $F(2, 40) = 0.556$, $\eta^2 = 0.027$, $p = 0.578$; sex factor: $F(1, 40) = 0.633$, $\eta^2 = 0.016$, $p = 0.431$, genotype x sex interaction: $F(2, 40) = 0.946$, $\eta^2 = 0.045$, $p = 0.397$; frequency: duration: genotype factor: $F(2, 40) = 0.292$, $\eta^2 = 0.014$, $p = 0.748$; sex factor: $F(1, 40) = 0.482$, $\eta^2 = 0.012$, $p = 0.492$, genotype x sex interaction: $F(2, 40) = 0.361$, $\eta^2 = 0.018$, $p = 0.699$, Fig. 13A, B). A third trial was conducted one week later to ensure that the preference for the novel arm was not driven by a natural preference for one of the side arms. In this trial, mice spent equal amounts of time in the left and right arms, indicating that they did not have a natural side preference (Arm: $F(1,40) = 0.844$, $\eta^2 = 0.021$, $p = 0.364$, Arm x genotype: $F(2,40) = 0.758$, $\eta2 = 0.037$, $p = 0.475$). Hence, *Nrxn1α* KO and HET mice do not display impaired short-term spatial memory.

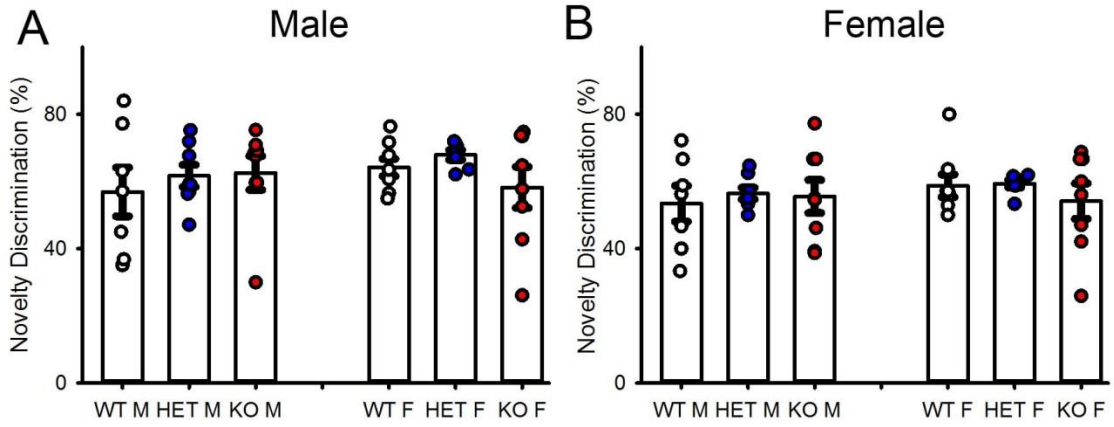

Figure 13: Short-term spatial memory in *Nrxn1α* WT, HET and KO mice.

A: Mean ± sem novelty discrimination calculated from duration in the novel and familiar choice arms in WT, HET and KO mice with overlaid scatter. B: Mean ± sem novelty discrimination calculated from number of entries into the novel and familiar choice arms in WT, HET and KO mice with overlaid scatter. Numbers of mice: WT M = 7, WT F = 8, HET M = 8, HET F = 7, KO M = 8, KO F = 8.

2.3.4.2 Morris Water Maze

Long-term spatial memory was assessed in a Morris Water Maze. All mice displayed a significant reduction in the time taken to reach the platform across days (Day factor: $F(6, 240) = 10.984$, $\eta^2 = 0.215$, $p < 0.001$). In addition, mice showed a significant reduction in distance travelled to reach the platform (path length) across days (Day factor: $F(6, 240 = 13.190$, $\eta^2 = 0.248$, $p < 0.001$). Thus, all mice displayed significant learning of the platform location. Female mice displayed significantly shorter path lengths than male mice (sex x day factor: $F(6, 240 = 3.122$, $\eta^2 = 0.072$, $p = 0.06$, sex factor: ($F(1, 40) = 1.799$, $\eta^2 = 0.119$, $p = 0.025$) indicating faster learning in females. There was no significant effect of genotype in either the time taken to reach the platform (genotype: $F(2,40) = 2.190$, $\eta^2 = 0.099$, $p = 0.125$,

Fig. 14A, B) or path length (genotype: $F(2,40) = 1.796$, $\eta^2 = 0.082$, $p = 0.179$, Fig. 14C, D). This indicated that all mice learnt the task at a similar speed.

A probe trial was conducted on the last day of spatial learning to test memory for the location of the platform. In this trial, the platform was removed from the maze. The time spent in the four different quadrants of the maze was measured. Mice spent significantly longer in the quadrant previously containing the platform than in the other three platforms (quadrant factor: $F(3, 120) = 29.444$, $\eta^2 = 0.424$, $p < 0.001$, Fig. 14E, F) but there were no significant genotype or sex effects. Thus, all mice displayed long-term memory for the platform location, and KO and HET mice did not display impairments in long-term spatial memory.

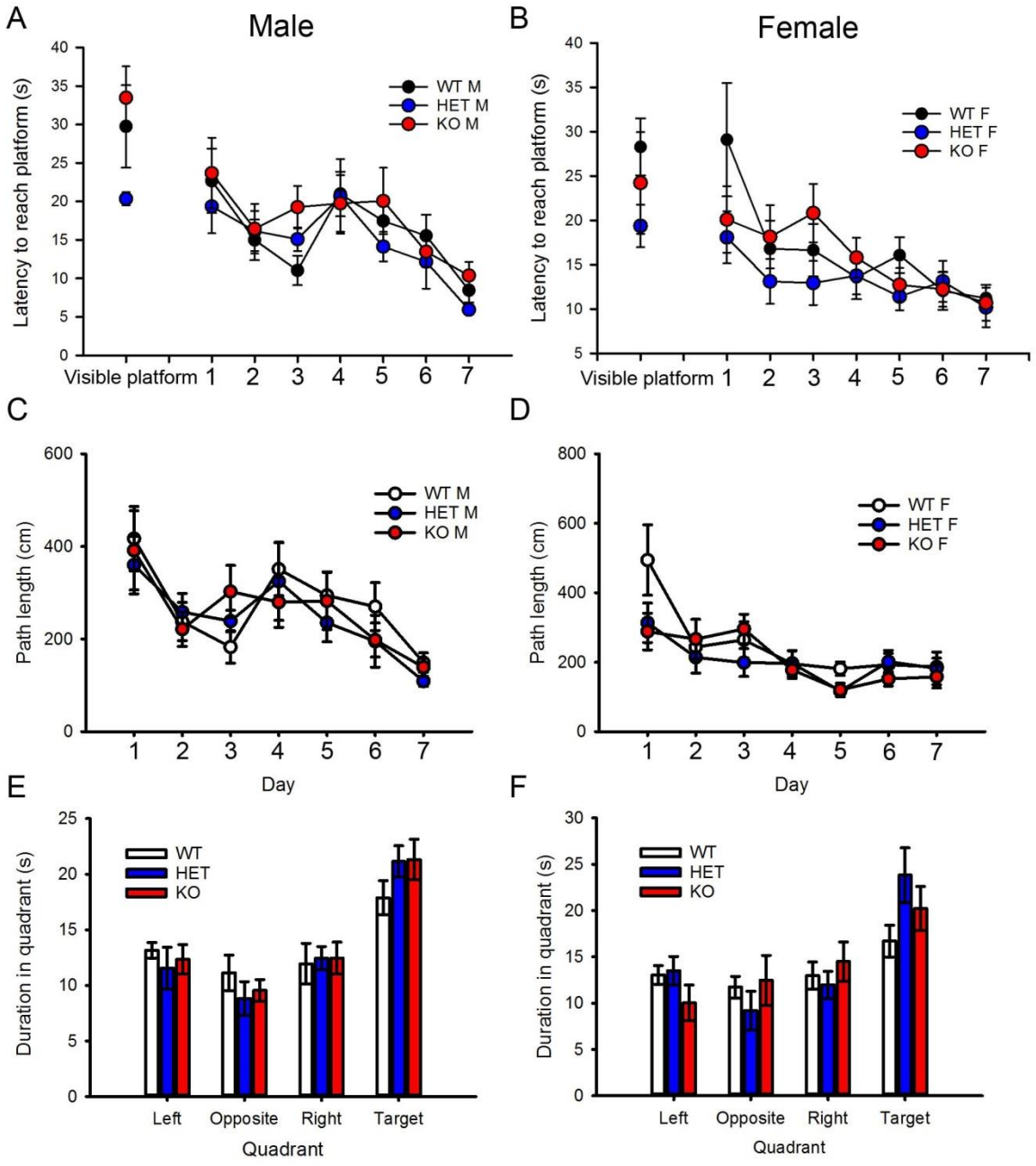

Figure 14: Intact long-term spatial memory in *Nrxn1α* mice.

A, B: Mean ± sem latency to reach the platform across training days in male (A) and female (B) WT, HET and KO mice. C, D: Mean ± sem path length to reach the hidden platform across training days in male (C) and female (D) WT, HET and KO mice. E, F: Mean ± sem time spent in each quadrant of the water maze on the probe trial in male (E) and female (F) WT, HET and KO mice. Numbers of mice: WT M = 7, WT F = 8, HET M = 8, HET F = 7, KO M = 8, KO F = 8.

2.3.5 Intact executive functions in *Nrxn1α* KO mice

Executive dysfunction is a common symptom in autism spectrum disorders, schizophrenia, and various other neurodevelopmental disorders. Therefore, I sought to investigate whether a microdeletion in *Nrxn1α* affected executive function by assessing reversal learning and problem-solving.

2.3.5.1 Reversal learning

Reversal learning was assessed in the Morris Water Maze by switching the location of the hidden platform to the opposite platform once all mice had learned the previous platform location. All mice displayed significant learning of the new platform location across training days, as measured by a reduction in the time taken to reach the platform and a reduction in the distance travelled to reach the platform (latency to platform, day factor: $F(4, 160) = 30.187$, $\eta^2 = 0.430$, $p < 0.001$; path length, day factor: $F(4, 160) = 25.029$, $\eta^2 = 0.385$, $p < 0.001$). There were no significant effects of genotype in either latency to reach the platform ($F(2,40) = 0.739$, $\eta^2 = 0.036$, $p = 0.484$, Fig. 15A, B) or path length ($F(2,40) = 0.109$, $\eta^2 = 0.005$, $p = 0.897$, Fig. 15C, D). In a probe trial conducted on the last day of testing, all mice spent significantly more time in the new platform location than in the other three quadrants (quadrant factor: $F(3, 120) = 50.297$, $\eta = 0.557$, $p < .001$, Fig. 15E, F).

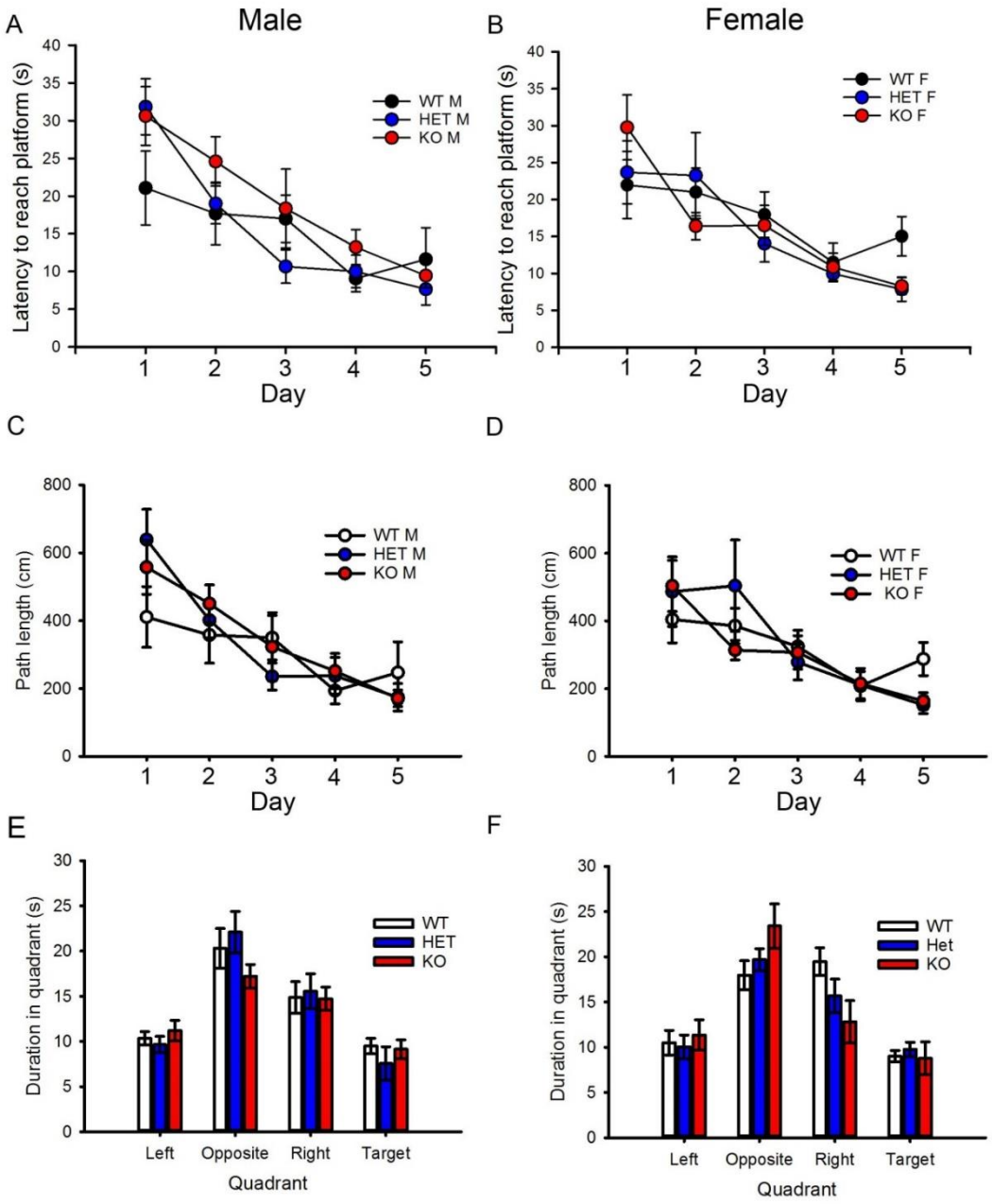

Figure 15: Reversal learning in the Morris Water Maze task.

A, B: Mean ± sem latency to reach the new platform location across training days in male (A) and female (B) WT, HET and KO mice. C, D: Mean ± sem path length to reach the new platform location (opposite platform) across training days in male (C) and female (D) WT, HET and KO mice. E, F: Mean ± sem time spent in each quadrant of the water maze on the reversal probe trial in male (E) and female (F) WT, HET and KO mice. Numbers of mice: WT M = 7, WT F = 8, HET M = 8, HET F = 7, KO M = 8, KO F = 8.

2.3.5.2 Puzzle box

Problem-solving abilities were assessed using the puzzle box. *Nrxn1α* WT, HET and KO displayed similar latencies to solve the puzzles on the first trial of every new puzzle, with the exception of puzzle 3, where KO mice performed significantly faster, and puzzle 1, where HET mice performed slower (Genotype x Puzzle factor: $F(6,147) = 3.569$, $\eta^2 = 0.127$, $p = 0.002$; Bonferroni-corrected t-tests, puzzle 3: KO vs WT: $p = 0.001$, KO vs HET: $p = 0.006$, puzzle 1: HET vs WT: $p = 0.020$, Fig. 16A, B). With repeated practice on each puzzle, differences emerged in latency to perform the puzzles; Unexpectedly, WT and HET mice became slower at reaching the "goal" box, suggesting reduced motivation to perform the task. In contrast, KO mice became faster at solving the puzzles (Genotype factor: $F(2,49) = 17.434$, $\eta^2 = 0.416$, $p < 0.001$; Bonferroni-corrected t-tests: KO vs WT: $p < 0.001$, KO vs HET: $p < 0.001$). This suggested that KO mice were more motivated to escape the aversive "start" area and access the "goal" box. Since bright light is anxiogenic to mice, I hypothesised that this may relate to the heightened anxiety observed in KO mice in the open field test.

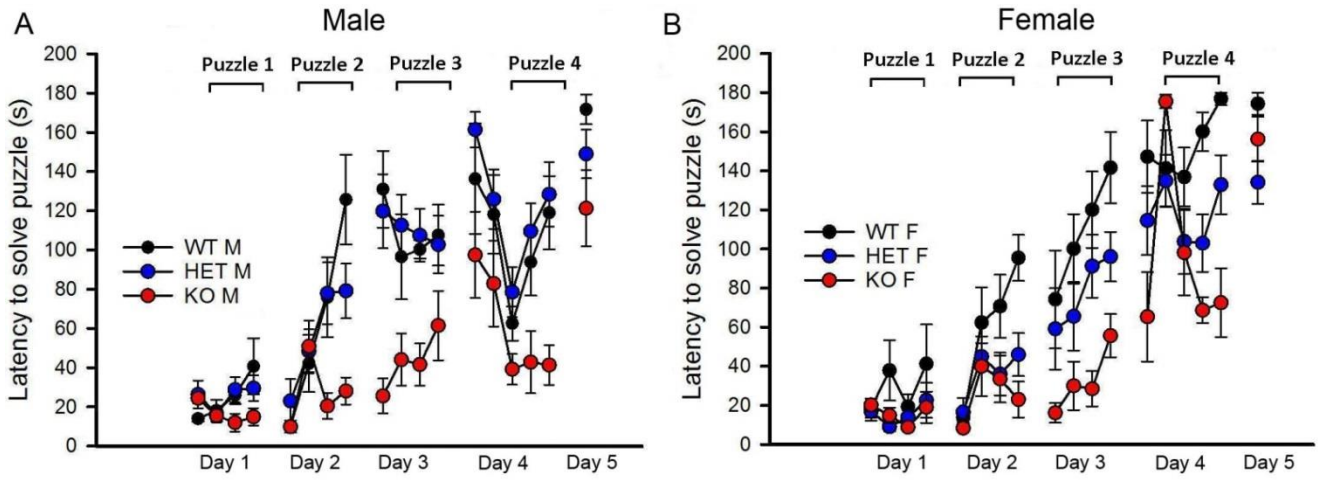

Figure 16: Problem-solving behaviour in the puzzle box task.

A, B: Mean ± sem latency to solve puzzles across puzzles and days in male (A) and female (B) *Nrxn1α* WT, HET and KO mice. Each day consists of a memory trial for the puzzle presented on the preceding day, followed by three trials of a new puzzle. Numbers of mice: WT M = 7, WT F =8, HET M = 12, HET F = 12, KO M = 10, KO F = 6.

2.4 Discussion

Impaired social cognition is observed across numerous neurological and psychiatric conditions, and is particularly prevalent in conditions that arise during development. Difficulties interpreting social cues and responding appropriately during social interactions are common features of social dysfunction. However, the neural mechanisms underlying these skills are unknown. Whilst attempts have been made to dissect the neural circuitry involved in social behaviour in rodent models, these have largely concentrated on mechanisms controlling the motivation to interact (social approach), rather than those underlying other aspects of dysfunctional social interaction.

The aim of this study was to examine the *Nrxn1α* KO mouse model as a potential model to investigate the circuitry underlying dysfunctional regulation of social behaviour. The *Nrxn1α* microdeletion is a risk factor for multiple neurodevelopmental disorders. In a previous study, homozygote microdeletions in murine *Nrxn1α* caused aggression and disinhibition of social behaviour (Grayton et al., 2013), suggesting that this mouse line may be a good candidate to study dysfunctional social control. In this study, I sought to replicate and further investigate the social phenotype of *Nrxn1α* KO mice in order to explore its validity as a model of social dysregulation. Moreover, I aimed to assess the specificity of the phenotype to social cognition by investigating whether non-social cognition was also impaired in these mice. My results showed that *Nrxn1α* KO mice display abnormally heightened social approach,

replicating the results from Grayton et al 2013. This indicates that the *Nrxn1α* KO mouse phenotype is robust. In addition, I found that *Nrxn1α* KO mice do not display cognitive impairment, indicating that social dysfunction is not secondary to a generalised cognitive deficit. These results demonstrate that the phenotype is specific to social behaviour and suggests that the *Nrxn1α* KO mouse is a promising model to study dysregulated social cognition.

My results indicate that *Nrxn1α* KO mice display abnormally heightened social approach in the three-chamber task. This is in agreement with earlier reports using the same mouse line and genetic background (Grayton et al., 2013). This phenotype was not observed in earlier studies of mice maintained on a mixed genetic background (Etherton et al., 2009). However, maintaining mice on a mixed genetic background can confound analysis of phenotype because genetic background effects contribute to phenotype and are variable across mouse strains (Koike et al., 2006). My results lend support to those of Grayton et al 2013, suggesting that the phenotype is robust, and that the failure to observe this phenotype in earlier studies arose from the confounding effect of maintaining mice on a mixed genetic background.

There are a number of possible explanations for the heightened social approach seen in *Nrxn1α* KO mice. This heightened social approach does not appear to reflect a positive, adaptive change in social cognition, since KO mice display significantly increased aggression in social interaction tasks (Grayton et al., 2013). One possibility is that longer exploration of social cues is driven by the mice taking longer to process social cues. Since *Nrxn1α* KO mice also display heightened aggression, an alternative explanation is that it is driven by this increase in aggression. One difficulty with this interpretation is that only male KO mice display aggression, whereas both female and male KO mice display heightened social approach. However, this may result from the fact that aggression is not a prominent feature of

the female mouse behavioural repertoire. It is also worth noting that these two interpretations are not mutually exclusive; a failure to detect and/or appropriately interpret social cues may underlie the increased aggression observed in direct social interaction tasks. This notion is supported by the fact that *Nrxn1α* KO mice display aggression towards social stimuli that do not usually elicit aggression (juvenile mice), as well as adult mice.

The ability to recognise conspecifics is a simple form of social cue recognition. Impairments in the ability to recognise social cues may impair social recognition memory. In an earlier study, *Nrxn1α* KO mice did not show an impairment in social recognition (Grayton et al., 2013). However, a deficit may be revealed under more taxing testing conditions. Social recognition can be made more taxing by the addition of a short delay during which mice have to maintain and retrieve relevant social information. In my experiments, *Nrxn1α* KO mice did not display reduced preference for a novel conspecific over a familiar conspecific after a short delay, although no male mice displayed evidence of social recognition memory. The absence of a deficit in female KO mice suggests that *Nrxn1α* does not influence the ability to process basic social cues required to recognise and remember conspecifics. However, interpretation of this task is inherently limited, because no male mice showed any preference for the novel conspecific. One past study has suggested that microdeletions in *Nrxn1α* impair social memory; in this study, *Nrxn1α* HET mice exhibited impaired social memory for familiar versus novel conspecifics in a three-chamber task (Dachtler et al., 2014).

The ability to recognise conspecifics relies on simple social perceptive abilities to pick up information about social identity. *Nrxn1α* KO mice have previously been shown to have normal olfactory ability (Grayton et al., 2013). Social interaction, however, requires more than simply recognising conspecifics. The social behaviours of conspecifics must also be correctly interpreted to identify the nature of the social interaction (for e.g. is a situation

threatening positive?). This requires understanding different types of social behaviour and attributing an emotional valence (positive or negative) to the behaviour. Sexual cues are one type of social cue that produce an affective response. In adult male mice, female urine or interaction with females evokes ultrasonic vocalisations (USVs) in the 50 kHz range, whilst male urine or interaction with other males evokes little response. These distinctive USVs are produced in rewarding contexts, and appear to elicit social approach. Therefore, they function as both a meaningful social cue, and a response to social cues. Thus it is possible to use USVs to measure both whether mice are able communicate, and probe into the valence associated with social cues. In my experiments, I found that *Nrxn1α* KO and WT mice made a similar number of 50-kHz USVs in response to interaction with a female mouse. This suggests that *Nrxn1α* KO mice do not possess a deficit in ultrasonic communication. In addition, this result indicates that despite their altered behaviour in social situations, *Nrxn1α* KO mice do find at least some kinds of social behaviour rewarding. This appears to support the notion that the aggression displayed by *Nrxn1α* KO reflects inappropriate behavioural responding rather than an impaired ability to assess the valence of social interaction.

Impaired social cognition in neurodevelopmental disorders can involve deficits across multiple facets of social cognition. In addition, different individuals with the same disorder can present with distinct social profiles. The majority of mice carrying genetic risk factors for neurodevelopmental disorders display impairments in social approach and social motivation (Goorden et al., 2007; Moy et al., 2009; Sato et al., 2012; Dachtler et al., 2014; Born et al., 2015b; Katayama et al., 2016; Jamain et al., 2008; Bozdagi et al., 2010; Won et al., 2012). However, not all individuals with impaired social cognition display reduced social motivation. In fact, in certain individuals, social motivation is intact, or even heightened. For example, some individuals with autism spectrum disorder display heightened social interest (Wing & Gould, 1979). Moreover, individuals with William's syndrome display heightened

sociability (Davies et al., 1998; Jones et al., 2000). Importantly, despite intact social motivation, these individuals nonetheless display impairments in the quality of their social interactions; often, these individuals engage in behaviours that are perceived as abnormal or even "offputting" to others (Wing & Gould, 1979). The altered social behaviour displayed by *Nrxn1α* KO mice displays some similarity to this phenotype. Indeed, these mice display heightened motivation and intact social reward processes, but exhibit inappropriate aggression during social interaction. This phenotype has rarely been observed in other mouse models. Hence, *Nrxn1α* KO mice may be a good model in which to study facets of impaired social behaviour that are not modelled in other mouse models.

A second aim of this study was to investigate whether the abnormal social behaviour seen in *Nrxn1α* KO mice is accompanied by non-social cognitive deficits. Cognitive deficits are often observed in neurodevelopmental disorders. For example, individuals with autism spectrum disorders often display a rigid, inflexible cognitive style, characterised by poor executive functioning (Hill, 2004). Cognitive deficits, including impaired working memory, are also common in schizophrenia (Nuechterlein et al., 2004; Green, Kern and Heaton, 2004b; Breton *et al.*, 2011). In addition, individuals with neurodevelopmental disorders often present with comorbid intellectual disability (Morgan, Leonard, Bourke, & Jablensky, 2008b).

Intellectual disability and cognitive impairments have been reported in some carriers of the human *Nrxn1α* microdeletion (Stefansson et al., 2013). However, the *Nrxn1α* microdeletion displays incomplete penetrance for neurodevelopmental disorders, suggesting that other genetic and environmental factors may contribute to and influence the phenotype of carriers. Hence, it is unclear to what extent cognitive impairments are being driven by these other factors, or by an interaction between the *Nrxn1α* microdeletion and other contributing factors. In this study, *Nrxn1α* KO and HET mice displayed no cognitive impairments when tested in

an extensive cognitive battery. An absence of cognitive impairment has also been reported in other studies investigating the *Nrxn1α* mice (Etherton et al., 2009; Grayton et al., 2013), although one study reported an altered response to novelty in *Nrxn1α* heterozygote mice (Laarakker, Reinders, Bruining, Ophoff, & Kas, 2012). Overall, these results suggest that the *Nrxn1α* microdeletion is not sufficient to cause cognitive impairment. There may be several reasons why a cognitive impairment has been observed in some human carriers of the microdeletion, but not in mice carrying the heterozygote or homozygote deletion. First, it may reflect a difference in the severity of the effect of the behavioural consequences of the microdeletion in mice compared to humans. The human *Nrxn1α* gene may possess additional functions that are not present in mice, and that cannot be compensated for by other related molecules (e.g. NRXN1β, NRXN2 or NRXN3). This notion is supported by the finding that heterozygote deletion of *Nrxn1α* is not sufficient to evoke any impairment in social, cognitive or explorative behaviour in mice, whilst it is the heterozygote microdeletion that is associated with neurodevelopmental disorders in humans (the homozygote deletion being associated with a more severe phenotype). The fact that cognitive impairments have also been reported in rats carrying the *Nrxn1α* microdeletion also appears to support this suggestion, although it is unclear whether this phenotype is mediated by anxiety (Esclassan, Francois, Phillips, Loomis, & Gilmour, 2015).

An alternative possibility is that the human *Nrxn1α* microdeletion is not sufficient to generate cognitive impairments. Instead, cognitive impairments may only emerge through interaction with other genetic or environmental factors is required. Indeed, many neurodevelopmental disorders are polygenic, and are thought to require an interaction between multiple risk factors to elicit disease. For example, a 'two-hit' model has been proposed in schizophrenia (Maynard, Sikich, Lieberman, & LaMantia, 2001). In the case of the NRXN1, the microdeletion shows incomplete penetrance, and interactions with other factors is likely

required to cause disorders of neurodevelopment. It would be of interest to examine the effects of combining the murine *Nrxn1α* mutation with other risk factors for neurodevelopmental disorders.

A third explanation is that the *Nrxn1α* gene plays a negligible role in non-social cognition, with other factors entirely accounting for cognitive deficits in patients. Indeed, autism and schizophrenia are neurodevelopmental disorders with a complex aetiology, making it unlikely that one gene would recapitulate the full disorder. Whilst the presence of cognitive deficits in the *Nrxn1α* KO rat argue against this explanation, it is possible that cognitive impairment in the rats reflected a secondary consequence of heightened anxiety, rather than a primary cognitive deficit. Indeed, anxiety was not measured or controlled for in the aforementioned study, although microdeletions in *Nrxn1α* have been associated with increased anxiety (Grayton et al., 2013; Viñas-Jornet et al., 2014). Other support for this suggestion comes from recent genetic studies exploring the aetiology and clustering of symptoms in autism spectrum disorders. In these studies, different symptom domains (e.g. social symptoms, cognitive deficits, repetitive behaviours) were found to be mediated by distinct genetic factors (Ronald, Happe & Plomin, 2005; Ronald *et al.*, 2006).

The lack of cognitive phenotype in the presence of a social phenotype is interesting. Whilst cognition is substantially impaired in some neurodevelopmental disorders, there are also conditions in which cognition is largely spared but social cognition is profoundly altered. In addition, there is evidence that social cognition is particularly susceptible to disruption. For example, the effects of brain injury acquired in development are substantially worse for social cognition than non-social cognition (Ryan et al., 2014). A key question is whether this reflects dissociation in the neural circuits and mechanisms underlying social and non-social cognition. This has been extensively debated in the literature. The idea that social cognition is

in some way "special", and that there may be dedicated neural circuits for social cognition has been a popular one (Adolphs, 2006; Brothers, 1996). A number of studies have suggested that there are regions of the brain that are preferentially involved in processing social information. For example, the fusiform gyrus appears highly selective for processing faces (Kanwisher et al., 1997). Likewise, the medial prefrontal cortex has been suggested to be particularly involved in social cognition (Amodio & Frith, 2006), although the specificity to processing social stimuli has been debated (Gauthier et al., 2000, 1999).

The presence of a social cognitive phenotype in the absence of a cognitive impairment in *Nrxn1α* KO mice suggests that there may be some dissociation in the mechanisms underlying social and non-social cognition. Whilst it is possible that *Nrxn1α* KO mice possess a sub-threshold, or undetected impairment in cognition, the fact that *Nrxn1α* KO mice displayed normal cognition across an extensive range of cognitive tasks argues against this. Genetic studies indicating that social and cognitive symptoms in autism have distinct genetic aetiologies and may be caused by different genetic risk factors support this idea (Ronald, Happe & Plomin, 2005; Ronald et al., 2006). However, an alternative possibility is that social cognition utilises shared mechanisms and circuits to non-social cognition, but that disruptions in social cognition are more difficult to compensate for.

In conclusion, I found that *Nrxn1α* KO mice display a robust and specific impairment in social behaviour. The presence of aggression and enhanced social approach suggests that these mice exhibit dysregulated social behaviour. The phenotype displayed by *Nrxn1α* KO mice suggests that these mice have some face validity as a model to study aspects of impaired social behaviour in neurodevelopmental disorders.

3 Excitatory circuitry in the orbitofrontal cortex of *Nrxn1α* KO mice

3.1 Introduction

3.1.1 The orbitofrontal cortex and social cognition

Multiple lines of evidence indicate that the orbitofrontal cortex performs functions that are vital for social cognition. Damage to the orbitofrontal cortex causes profound behavioural disturbances that affect social interactions. Symptoms displayed by affected individuals include heightened aggression and emotional reactivity, a tendency to engage in "inappropriate" behaviour, personality changes and reduced empathy. These changes in individual's abilities to engage in social interaction after orbitofrontal damage despite relatively intact non-social cognition have been reported both in case studies (Anderson et al., 2000; Cicerone & Tanenbaum, 1997; Eslinger & Damasio, 1985) and corroborated in larger studies (Grafman et al., 1996).

The role of the orbitofrontal cortex in dysfunctional social cognition has been further explored using quantitative tests of social cognition. In such tests, individuals with damage to the orbitofrontal cortex display difficulties across a range of social tasks. These include deficits in the ability to recognise facial expressions, difficulty predicting the consequences of social behaviour, impaired ability recognise social "rules" and "faux pas", and deficits in moral judgements (Ciaramelli, Muccioli, Làdavas, & di Pellegrino, 2007b; Mah et al., 2004; Stone et al., 1998; Zahn et al., 2017b). In contrast, non-social cognition is often largely intact in these individuals, although difficulties with certain aspects of decision-making and reward processing are reported (Anderson et al., 2000).

The importance of the orbitofrontal cortex in social cognition has also been supported by blood-oxygen-level dependent (BOLD) functional magnetic resonance (fMRI) neuroimaging studies. These studies demonstrate that the orbitofrontal cortex is involved in a number of facets of social cognition, including emotion perception, regulation of aggression behaviour, and recognition of "appropriate" social behaviour. For example, the medial orbitofrontal cortex is highly active during emotional perspective-taking theory of mind, suggesting a role in emotion perception and understanding (Hynes et al., 2006). BOLD activity in the orbitofrontal cortex during viewing of an angry facial expression is negatively correlated with aggressive behaviour during social interaction, indicating a role in regulating aggression (Beyer et al., 2015). In addition, the orbitofrontal cortex is part of a network of regions activated during reading violations of social norms (Bas-Hoogendam et al., 2017).

The orbitofrontal cortex also plays a role in the regulation of social behaviour in non-human primates and rodents. As in humans, lesions to the orbitofrontal cortex lead to heightened aggression in non-human primates (Machado & Bachevalier, 2006). Similarly, rats with bilateral orbitofrontal cortex lesions display increased aggression, which is mimicked by dopamine depletion in the orbitofrontal cortex (De Bruin, 1990; De Bruin et al., 1983). In contrast, injection of 5-HT1B agonists into the ventral orbitofrontal cortex reduces aggression in the resident-intruder paradigm in mice (De Almeida et al., 2006). Moreover, evidence indicates that the orbitofrontal cortex is important for recognition of social signals and social contexts in monkeys and rodents. Monkeys with orbitofrontal lesions demonstrate altered response to affiliative and threatening signals (Machado & Bachevalier, 2006). Acetylcholinergic input in the orbitofrontal cortex is necessary for the acquisition of socially transmitted food preferences in rats (Ross, McGaughy, & Eichenbaum, 2005). In rats, orbitofrontal lesions impair the ability to modulate social interactions as a function of the context of the partner and can cause hyperactivity in play (Pellis et al., 2006).

In summary, converging evidence from human lesion, neuroimaging and rodent/non-human primate studies indicate that the orbitofrontal cortex is involved in the regulation of social behaviour. In particular, these studies indicate a role in the regulation of aggressive behaviour, as well as a role in recognising and responding to social cues and social contexts.

3.1.2 The orbitofrontal cortex in neurodevelopmental disorders

The symptoms observed as a result of orbitofrontal cortex damage bear resemblance to the impairments in social cognition described in individuals with neurodevelopmental disorders. For example, a tendency to engage in inappropriate behaviour, impaired theory of mind, and difficulties interpreting social signals have been observed in both individuals with neurodevelopmental disorders such as autism spectrum disorders and schizophrenia, and in individuals with orbitofrontal damage (Ashwin et al., 2006; Simon Baron-Cohen et al., 1985; Brune, 2005b; Frith & Corcoran, 1996b; Golan et al., 2007; Grafman et al., 1996; Happé, 1994; Hoptman et al., 2010b; Stone et al., 1998).

The similarity between social symptoms arising from orbitofrontal damage and those seen in neurodevelopmental disorders suggests that dysfunction of the orbitofrontal cortex may contribute to impaired social cognition in individuals with neurodevelopmental disorders. This suggestion has received some support from neuroimaging studies. For example, altered sulco-gyral patterns in the right orbitofrontal cortex and reduced volume of the orbital gyri have been observed in schizophrenia (Takayanagi et al., 2010). Both reductions in grey matter volume and changes in sulco-gyral patterns have been reported bilaterally in high-functioning individuals with autism spectrum disorder (Itahashi et al., 2015; Watanabe et al., 2014). In a separate study, decreased right orbitofrontal grey matter volume was described in children with autism, with social deficits correlating with right orbitofrontal white matter (Girgis et al., 2007). Finally, in one study, no global changes in orbitofrontal grey matter volume were observed in individuals with autism compared with controls, but preliminary

evidence was found for age-related changes in volume in the right lateral orbitofrontal in children with autism (Hardan et al., 2006).

Alterations in orbitofrontal cortex function and structure have also been described in a number of other neurodevelopmental conditions in which social cognition is impaired, including Turner's syndrome and William's syndrome. Females with Turner's syndrome have enlarged amygdala and orbitofrontal cortex volume, as well as deficits in recognising fearful facial expressions (Good et al., 2003). Individuals with William's syndrome display inverted patterns of BOLD activation in the medial and lateral right orbitofrontal cortex in response to positive and negative faces (Mimura et al., 2010), as well as reduced BOLD activation in the orbitofrontal cortex during face-matching tasks using threatening faces (Meyer-Lindenberg et al., 2005). Finally, individuals with William's syndrome display altered grey matter structure in the lateral orbitofrontal cortex, as well as the lingual gyrus, post central gyrus and cuneus (Meda, Pryweller, & Thornton-Wells, 2012).

3.1.3 Animal studies of the circuitry underlying social cognition

The circuits underlying social behaviour have begun to be explored in more detail in rodent models. Much of the work undertaken so far has concentrated on the circuitry controlling social approach via optogenetics. These studies have revealed that circuits involving the basolateral amygdala, ventral hippocampus, ventromedial prefrontal cortex and dorsal raphe nucleus are involved in controlling approach and motivation for social contact. For example, optogenetic activation and inhibition of connections between the basolateral amygdala and ventral hippocampus reduce, and increase social approach, respectively (Felix-Ortiz & Tye, 2014). In contrast, optogenetic activation of excitatory projections from the ventromedial prefrontal cortex/orbitofrontal cortex to the dorsal raphe nucleus increase approach after social defeat (Challis et al., 2014). Moreover, elevating the excitatory/inhibitory balance in

the medial prefrontal cortex of the mouse reduces social interaction (Yizhar, Fenno, *et al.*, 2011).

Optogenetics has also been used to explore circuitry mediating aggression. Circuitry within the ventromedial hypothalamus is important for the expression of rodent aggressive behaviours. Specifically, the dorsomedial division of the ventromedial hypothalamus is involved in defensive aggression upon exposure to a predator, whereas the ventrolateral ventromedial hypothalamus controls the expression of aggression towards conspecifics (Silva et al., 2013). Interestingly, optogenetic activation of the ventromedial hypothalamus increases aggression in male mice both within the context of a resident-intruder paradigm, and within contexts in which male mice do not normally display aggression e.g. towards female mice (Falkner, Grosenick, Davidson, Deisseroth, & Lin, 2016; Lin et al., 2011). Other brain regions provide "top-down" control over aggressive behaviours by projecting to the ventrolateral ventromedial hypothalamus. For example, projections from the lateral septum to the ventrolateral ventromedial hypothalamus inhibit aggressive bouts (Wong et al., 2016). The prefrontal cortex also provides 'top-down' control over aggression; activation of the prefrontal cortex inhibits inter-male aggression in a resident-intruder task (Takahashi et al., 2014).

In contrast, the orbitofrontal cortex has received little optogenetic investigation. Numerous studies have explored how neuronal activity in the orbitofrontal cortex contributes to non-social cognition using *in vivo* electrophysiology (Saez et al., 2017; Schoenbaum et al., 1999; Schoenbaum & Eichenbaum, 1995a; Sul, Kim, Huh, Lee, & Jung, 2010; Tremblay & Schultz, 2000). However, few studies have investigated how manipulation of orbitofrontal circuitry affects social behaviour in rodent model, despite evidence from lesion and neuroimaging studies indicating that the orbitofrontal cortex is important for social cognition.

3.1.4 Excitatory circuitry disturbances in neurodevelopmental disorders

Numerous lines of evidence suggest that excitatory circuitry may be disturbed in disorders of neurodevelopment. Evidence that excitatory circuitry might be abnormal first came from neuropathology studies investigating the number of dendritic spines in the brains of individuals with neurodevelopmental disorders (Glantz and Lewis, 2000; Irwin et al., 2001; Hutsler and Zhang, 2010). This has been accompanied by reports of changes in the expression of genes encoding proteins that are involved in excitatory synaptic function (Harrison & Eastwood 1998; Purcell et al 2001), and altered plasma and serum glutamate levels in individuals with neurodevelopmental disorders (Shinohe et al., 2006).

Studies investigating genetic risk factors for neurodevelopmental disorders also support the notion that excitatory circuitry is disturbed in some neurodevelopmental disorders. Many genetic mutations, deletions, and abnormalities implicate genes involved in synapse formation and function (Bourgeron, 2009). This includes mutations and risk variants in genes encoding pre-synaptic proteins such as neurexins and CNTNAP2, as well as in post-synaptic proteins such as neuroligins and SHANK proteins (Alarcón et al., 2008; Arking et al., 2008; Durand et al., 2007a; Fromer et al., 2014; Gauthier et al., 2009, 2010, 2011; Jamain et al., 2003; Kim et al., 2008; Laumonnier et al., 2004; Strauss et al., 2006; Vaags et al., 2012). Many of these genes encode proteins that are play a primary role at excitatory synapses. For example, alterations in the Glutamate receptor 6 gene have been linked to ASD (Jamain et al., 2002), and SHANK proteins are present at glutamergic synapses. Electrophysiological and structural studies of mice carrying genetic risk factors of neurodevelopmental disorders also broadly support this idea (Born et al., 2015b; Dani & Nelson, 2009; Etherton et al., 2009a; Harrington et al., 2016; Jiang & Ehlers, 2013b; Qiu et al., 2011; Won et al., 2012), although see Peñagarikano et al., 2011 and Tabuchi et al., 2007 for mouse models in which deficits were predominantly seen in inhibitory circuitry.

These findings have led to the proposal that neurodevelopmental disorders may be caused by deficits in synaptic pruning. The nature of such a deficit is unclear; both hyperconnectivity/impaired synaptic pruning and hypoconnectivity/excessive synaptic pruning have been suggested. The direction of change may depend on the condition. Studies of individuals with schizophrenia suggest that synaptic density is reduced across certain brain regions including the prefrontal cortex (Glantz & Lewis, 2000) and medial temporal cortex (Harrison & Eastwood, 1998). In contrast, increased spine density has been reported in the brains of individuals with autism (Hutsler & Zhang, 2010). Hyperconnectivity has also been reported in the visual and temporal lobes of individuals with Fragile X syndrome (Irwin et al., 2001). Intriguingly, many of such studies have observed changes in spine density predominantly in cortical layers 2/3.

Whilst there is evidence of abnormal excitatory synapse density in neurodevelopmental disorders, there are also some difficulties with this theory. The direction of changes in excitatory function is not always consistent across studies. For example, decreased plasma levels of glutamate were described in patients with autism in one study (Aldred, Moore, et al., 2003), but a separate study found increased serum levels of glutamate in individuals with autism (Shinohe et al., 2006).

There is also variability in results in animal studies investigating mice carrying genetic risk factors for neurodevelopmental conditions. A number of studies of mice carrying autism risk-factor mutations or mutations that cause syndromic autism suggest that excitatory connectivity is reduced. For example, reduced excitatory synaptic function has been observed in somatosensory cortex of mice carrying a mutation in MeF2c that causes syndromic autism, (Harrington et al., 2016). Likewise, mice carrying a mutation in the methyl CpG binding protein 2 (MeCP2) gene, which causes Rett syndrome in humans, display reduced mEPSC

frequency in somatosensory cortex in MeCP2 mutant mice (Dani et al., 2005) and reduced pyramidal neuron connectivity (Dani & Nelson, 2009). It is worth noting that these studies used male mice, and may only recapitulate certain features of Rett syndrome. Indeed, MeCP2 is located on the X chromosome, and in humans Rett syndrome predominantly affects females because males die at birth. However, layer-specific excitatory input reductions have also been reported when MeCP2 is knocked down from subsets of cells in wild-type mice (Wood, Gray, Zhou, Greenberg, & Shepherd, 2009).

In contrast, increased connectivity has also been reported in certain rodent models. This includes mice carrying a mutation in the MET receptor tyrosine kinase gene (MET), which is also a candidate gene associated with sporadic ASD. Conditional deletion of MET in mice leads to increased synaptic connectivity in corticostriatal neurons in frontal cortex (Qiu et al., 2011). Increased spine density and abnormal spine shapes have also been reported in FMRP KO mice (Greenough et al., 2001). Finally, rats treated *in utero* with valproic acid also display local circuit hyperconnectivity, although this study also found that excitatory synaptic strength was reduced (Rinaldi, Silberberg, & Markram, 2008).

Hence, the findings are mixed. The direction of changes in excitatory synapse number is not always consistent across studies. In addition, some mouse models of neurodevelopmental disorders fail to find any change in synapse density (Born et al., 2015b; Etherton et al., 2009a). Neuropathological studies also suffer from limitations; both sample sizes and effect sizes are typically small in such studies, and the findings are not consistent across all individuals. This may partly reflect heterogeneity in the causes of neurodevelopmental disorders. Indeed, other abnormalities have also been proposed in neurodevelopmental disorders, including dysfunction of inhibitory GABAergic neurotransmission, or a disrupted excitatory/inhibitory balance (Rubenstein & Merzenich, 2003). Lastly, changes in synapse

number have not been found across all brain regions in individuals with neurodevelopmental brain regions, despite the fact that these conditions may affect widespread brain development. Indeed, in some cases, no changes in synapse density have been found in brain regions that nonetheless display abnormal responses in fMRI BOLD imaging studies or in MRI grey/white matter structural imaging.

This suggests that interpreting neurodevelopmental disorders as disorders of synaptic density may be overly simplistic. Other forms of circuit disruption are likely to contribute in many cases. It is possible that changes in synapse density are a present in only certain individuals, or in only certain brain regions or pathways.

3.1.5 Disruption of short and long-range connectivity

An extension of the synaptic pruning and altered synaptic connectivity theories of neurodevelopmental disorders is that changes in synaptic connectivity are pathway or region-specific. In particular, one idea that has gained prominence is the suggestion that these disorders are characterised an imbalance in local "short-range" and across brain region "long-range" connectivity. This theory has gained particular prominence in autism spectrum disorder research. Here, the most common formulation of this theory posits that local circuit connectivity is enhanced and long-range connectivity is reduced (Courchesne & Pierce, 2005).

MRI studies support the notion that long-range connectivity is altered in neurodevelopmental disorders. Reduced functional connectivity has been observed across diverse tasks and at rest in autistic individuals (Just et al., 2004; Kana et al., 2007; Koshino et al., 2005; Villalobos et al., 2005; Welchew et al., 2005). Diffusion tract imaging studies measuring water diffusion (fractional anisotropy) to map white matter tracts also lend support to this idea. These studies report reduced fractional anisotropy in white matter tracts, such as the corpus callosum, in

individuals with autism spectrum disorders, suggesting either reduced fiber density, axonal diameter or myelination in white matter tracts (Frazier & Hardan, 2009). Reduced white matter tract fractional anisotropy has also been reported in individuals with other neurodevelopmental disorders such as schizophrenia (Paillère-Martinot et al., 2001).

There are limitations to the support for this hypothesis from human imaging studies. Firstly, the findings are difficult to interpret. It is unclear whether or not functional connectivity changes reflect changes in anatomical connectivity (Leopold & Maier, 2012). Changes in fractional anisotropy are also subject to multiple interpretations, including both changes to the number of axons or altered myelination. In addition, changes in fractional anisotropy have not always consistently supported a decrease in long-range connectivity. Indeed, a number of studies have reported increased functional anisotropy in white matter tracts in individuals with autism spectrum disorders (Herbert et al., 2004; Radua, Via, Catani, & Mataix-Cols, 2011). Finally, imaging methods are limited in their ability to investigate local circuit connectivity. Findings of increased fractional anisotropy within the frontal lobes of individuals with autism have been interpreted as support for increased local connectivity (Ben Bashat et al., 2007), but could reflect long-range inputs. Increased spine density in cortical layers 2/3 in autistic individuals has also been considered indirect support for increased local connectivity, on the basis that local connections exceed long-range inputs. However, dendritic spines in layer 2 and 3 receive both local and long-range connections and there are multiple explanations for these findings; increased dendritic spines could reflect an imbalance of short and long-range connections or a global increase in the number of all connections.

Testing this hypothesis requires investigation of circuitry at the cellular level. A small number of studies have begun to explore this issue in mice carrying genetic risk factors for neurodevelopmental disorders. This requires looking beyond miniature excitatory post-

synaptic potentials (mEPSPs); mEPSPs provide only a global measure of excitatory synaptic function only, and cannot distinguish between local and long-range inputs. For example, in a study employing a combination of glutamate uncaging to stimulate local connections, and optogenetic stimulation of contralateral inputs, knock-down of Mef2c in a sparse population of somatosensory neurons reduced local synaptic connectivity, but enhanced excitatory input from contralateral somatosensory cortex (Rajkovich et al., 2017).

The majority of the focus in the investigation of short and long-range connectivity imbalances has concentrated on the number of connections. However, it is possible that other aspects of long and short-range connectivity are altered. For example, the strength, timing, and dynamics of long and short-range connections may be impaired. This cannot easily be studied with human imaging techniques. Instead, cellular electrophysiology is required to address these questions.

3.1.6 Long-range and short-range afferents to the orbitofrontal cortex

Long-range afferents to the orbitofrontal cortex have been investigated in monkeys and rodents using viral tracing. These studies have revealed that the orbitofrontal cortex receives projections from many different brain regions, including thalamic, limbic, temporal and sensory regions.

The mediodorsal thalamic nucleus is a major source of diencephalic afferents to the orbitofrontal cortex. The rat mediodorsal thalamic nucleus is subdivided into medial, central and lateral segments. The medial and central segments project to the orbitofrontal cortex. (Kuramoto et al., 2017). Inputs are also received from other deep brain structures, such as the dorse raphe nuclei, ventral tegmental area, hypothalamus and pontomesencephalic tegmentum (Morecraft et al., 1992; Reep et al., 1996).

Dense cortical inputs to the orbitofrontal cortex arise from the temporal lobes, insula and limbic brain regions. Temporal regions projecting to the orbitofrontal cortex include the entorhinal cortex and perirhinal cortex and hippocampus, posterior parahippocampal area as well as posterior parietal areas (Morecraft, Geula and Mesulam, 1992; Barbas, 1993; Carmichael and Price, 1995a). Limbic projections to the orbitofrontal cortex arise predominantly from the amygdala. Connections between the amygdala and orbitofrontal cortex are reciprocal and connect the orbitofrontal cortex with the basolateral amygdala, lateral amygdala and medial/central amygdala (Carmichael and Price, 1995a). These projections may be important for guiding goal directed behaviour on the basis of cued rewards (Lichtenberg et al., 2017).

The orbitofrontal cortex receives input from all sensory modalities. These sensory projections tend to project to lateral orbitofrontal areas (Morecraft et al., 1992). Projections from the olfactory bulb may play a role in linking odours to emotions (Tanabe et al., 1975). Inputs are also received from gustatory areas (Rolls & Baylis, 1994b). For example, the insular-opercular taste area projects to orbitofrontal cortex, where some neurons respond to gustatory stimuli (Baylis et al., 1995). Finally, inputs are also received from somatosensory, visual and auditory areas (Barbas, 1993; Rolls & Baylis, 1994b). These projections mainly arise from secondary sensory processing regions, such as the superior and inferior temporal sulcus (Kondo, Saleem, & Price, 2003). Similar results have been obtained in rat viral tracing studies (Reep et al., 1996).

Lastly, projections to the orbitofrontal cortex also arise from other prefrontal subregions, such as the medial prefrontal cortex and anterior cingulate cortex (Barbas, 1993; Carmichael and Price, 1995). Projections from the medial prefrontal cortex inhibit the pathway from lateral

orbitofrontal cortex to the basolateral amygdala (Chang & Ho, 2017), a pathway that is important for cue-outcome contingency learning.

In humans, a number of tracts have been identified that connect the orbitofrontal cortex to other brain regions. This includes a tract bundle connecting the orbitofrontal cortex with the thalamus and anterior cingulate cortex, a tract passing laterally through the caudate to reach the brainstem, and fibers passing through the inferior fronto-occipital fasciculus to connect the orbitofrontal cortex with parietal and occipital cortex. Some of these fibers turn and join the uncinate fasciculus to connect to superior and middle temporal lobes (Burks et al., 2017).

3.1.7 Long-range inputs to the orbitofrontal cortex and social cognition

Long-range projections to the orbitofrontal cortex are likely to play an important role in orbitofrontal regulation of social cognition. These connections provide input about the external world, and internal emotional states which are used to guide social decision-making.

Diffusion tractography imaging indicates that white matter projections to the orbitofrontal and prefrontal cortex are related to social abilities in adults and children. Increases in white matter within the prefrontal cortex and in the tracts connecting the temporoparietal and inferior frontal regions are associated with the development of theory of mind in children (Grosse Wiesmann, Schreiber, Singer, Steinbeis, & Friederici, 2017). Fractional anisotropy in the right uncinate fasciculus at 6 months is positively correlated with joint attention abilities in infants at 9 months (Elison et al., 2013). Moreover, fractional anisotropy within the uncinate fasciculus and frontal-dorsomedial thalamic tract correlate with emotional empathy (Parkinson & Wheatley, 2014). Finally, reduced fractional anisotropy within the uncinate fasciculus and inferior fronto-occipital fasciculus is related to poor social cognition in individuals carrying the 22q11.2 deletion (Jalbrzikowski et al., 2014).

Alterations to tracts projecting to the orbitofrontal cortex are associated with social cognitive impairments in a number of disorders. Alterations to the uncinate fasciculus, which connects the amygdala and the orbitofrontal cortex, affect social cognition. For example, damage to the uncinate fasciculus is associated with impaired sarcasm detection in individuals with behavioural variant fronto-temporal dementia (Downey et al., 2015). Individuals carrying the 22q11.2 microdeletion display altered white matter microstructure across numerous white matter tracts, including reduced fractional anisotropy in the uncinate fasciculus and decreased axial diffusivity in the inferior fronto-occipital fasciculus (Radoeva et al., 2012). Alterations in these tracts have also been reported in individuals with idiopathic schizophrenia (Ellison-Wright & Bullmore, 2009; Kuswanto, Teh, Lee, & Sim, 2012). In addition, impaired emotion recognition was associated with reduced fractional anisotropy in the inferior fronto-occipital fasciculus and corpus callosum in schizophrenic patients (Miyata et al., 2010). In contrast, individuals with William's syndrome display higher fractional anisotropy and axial diffusivity within the uncinate fasciculus and inferior fronto-occipital fasciculus (Haas et al., 2014). Increased white matter in the uncinate fasciculus, right arcuate fasciculus and left inferior fronto-occipital fasciculus has also been reported in autism spectrum disorders (Radua et al., 2011), as well as increased fractional anisotropy in the frontal lobe, cingulate gyrus, insula, superior temporal gyrus and cerebellar peduncle (Cheng et al., 2010). In addition, reduced gyrification of the inferior frontal lobe, inferior parietal region and medial parieto-occipital region was found to be related to reduced callosal functional anisotropy and lower inter-hemispheric frontal connectivity in ASD patients (Schaer et al., 2013).

Rodent studies suggest that the development and myelination of connections within the prefrontal cortex may be particularly important for social cognition. Mice that display social impairments as a result of social isolation during development display reduced myelination in the prefrontal cortex (Makinodan et al., 2016, 2012). In addition, functional connectivity

analyses reveal reduced connectivity within the orbitofrontal cortex of mice that are socially isolated during development (Liu et al., 2016a). These studies suggest that changes in connections within the prefrontal and orbitofrontal cortex may contribute to social cognitive impairments.

3.1.8 Mechanisms of vesicle release and miniature excitatory post-synaptic potentials

Multiple pre-synaptic proteins regulate pre-synaptic vesicle release. Pre-synaptic vesicle release arises when action potentials invade the pre-synaptic bouton and cause the influx of calcium. This signal activates fast synaptotagmin-1 calcium sensors which interact with SNARE proteins (vesicular SNARE, synaptobrevin; target membrane SNAREs, syntaxin-1 and SNAP-25) to trigger vesicle fusion and release of neurotransmitter.

Vesicle release at the pre-synaptic terminals comes from three different 'pools', including a 'rapid releasable pool' (RRP), a' recycling' pool and a larger 'reserve' pool (Rizzoli & Betz, 2005). Influx of calcium into the presynaptic bouton initiates the release of vesicles from the RRP. The readily releasable pool consists of the vesicles that are docked and primed for release. The 'recycling' pool is then released once the RRP is depleted. This pool is thought to be drawn upon during moderate physiological stimulation, when the RRP has run down. The reserve pool is rarely released during physiological stimulation, making it difficult to determine what role it plays.

Miniature EPSPs (mEPSPs) are due to the spontaneous release of single vesicles of neurotransmitter that occur in the absence of pre-synaptic action potentials. Fatt and Katz (1952) and del Castillo & Katz (1954) described these events at the neuromuscular junction and determined that these events showed similarity to end-plate potentials in waveform, localisation and response to drugs. They then showed that EPPs could be reduced to resemble miniature EPPs by incubating the synapses in low concentrations of calcium (del Castillo &

Katz, 1954). Furthermore, the EPP amplitudes could be predicted from the distribution of mEPP amplitudes. These studies led to the proposal that EPPs consisted of synchronous release of multiple 'mini-EPP', quantal events.

There has been some debate as to whether or not mEPSPs reflect release of a single vesicle or synchronised release of multiple vesicles. This debate has arisen due to the observation that mEPSPs vary considerably in amplitude (Korn and Faber, 1987), leading to the suggestion that mEPSPs reflect synchronised spontaneous release of vesicles caused by transient calcium influx. Whilst this synchronised release model predicts the variability and positive skew in the amplitude of mEPSPs, other research has demonstrated that this variability is retained when mEPSPs are evoked by internally perfused buffered calcium in the absence of external calcium or by non-calcium dependent means via alpha-latroxin (Frerking, Borges and Wilson, 1997). This supports the notion that mEPSP reflect release of a single vesicle.

mEPSPs provide a global measure of excitatory drive onto a post-synaptic neuron, and are therefore commonly used during investigation of excitatory circuit disturbances. However, measures of mEPSP frequency and amplitude do not enable determination of how specific inputs are altered.

3.1.9 Short-term facilitation

Short-term facilitation is an enhancement of the post-synaptic potential (PSP) response with repeated stimulation. This contrasts with short-term depression, where PSP amplitudes are reduced with repeated stimulation. Short-term facilitation is usually seen at synapses that initially have a lower probability of release and is thought to be predominantly controlled by pre-synaptic mechanisms, although there are a few cases in which a post-synaptic component has been described. For example, synapses with high levels of calcium permeable glutamate receptors can facilitate due to a postsynaptic mechanism (Andrei Rozov & Burnashev, 1999).

Normally, calcium permeable glutamate receptors are blocked by polyamines. High-frequency activation of calcium-permeable glutamate receptors releases the polyamine block and increases the postsynaptic response. This has been described at excitatory synapses onto multipolar interneurons (Andrei Rozov & Burnashev, 1999). However, it does not occur routinely at excitatory pyramid – pyramid synapses (Andrei Rozov & Burnashev, 1999).

Short-term facilitation is accompanied by a decrease in failures of the postsynaptic response and an increase in the number of quanta of neurotransmitter released, but no change in quantal amplitude (del Castillo & Katz, 1954). These findings suggest that short-term facilitation is due to more vesicles of neurotransmitter fusing with the pre-synaptic membrane rather than greater release of neurotransmitter by each vesicle.

A number of mechanisms in the presynaptic bouton have been suggested to contribute to short-term facilitation. One suggestion is that facilitation arises as a result of a larger calcium signal in the presynaptic axon terminal. The residual calcium hypothesis posits that the calcium concentration in the presynaptic terminal does not return to baseline levels before another action potential invades the presynaptic terminal (Katz & Miledi, 1968). This hypothesis requires a calcium sensor with slower kinetics than synaptotagmin-1 (syt1) or synaptotagmin (syt-2). Support for this idea comes from findings that lowering residual calcium attenuates facilitation at the crayfish neuromuscular junction (Kamiya & Zucker, 1994) and at Purkinje cell synapses (Atluri & Regehr, 1996). More recently, it has been demonstrated that residual calcium may contribute to facilitation via synaptotamin-7 (syt-7), a synaptotagmin with high calcium affinity and slower binding kinetics. Syt-7 KO mice display abolished paired-pulse facilitation and synaptic enhancement, with expression of WT syt-7 rescuing facilitation (Jackman, Turecek, Belinsky, & Regehr, 2016). However, whilst syt-7 can bind to SNAREs in a calcium-dependent manner (Sugita et al., 1999), it is unclear

whether syt-7 mediates facilitation by increasing the probability of syt-1 mediated vesicle fusion and release or by mediating release itself. Other molecules may also contribute to regulation of short-term facilitation and depression. For example, phosphorylation of SNAP-25 may play a role in down-regulating facilitation by regulating vesicle dynamics and release (Katayama et al., 2017).

Other mechanisms have also been proposed to contribute to facilitation. This includes action potential spike broadening, which increases pre-synaptic calcium entry, leading to greater facilitation (Jackson, Konnerth, & Augustine, 1991). This contributes to facilitation at mossy fiber synapses between granule and CA3 cells of the hippocampus (Geiger & Jonas, 2000). However, at many synapses, changes in action potential waveform are small, making this mechanism is unlikely to contribute to facilitation. In addition, use-dependent increases in calcium influx and saturation of calcium-binding proteins with repetitive stimulation have also been proposed to contribute to facilitation (Klingauf & Neher, 1997; Neher, 1998; Rozov, Burnashev, Sakmann, & Neher, 2001).

A number of studies investigating animal models of neurodevelopmental disorders have reported altered short-term plasticity. For example, cultured neurons from mice carrying a KO deletion of the MeCP2 gene display both reduced mEPSC frequency and increased short-term depression (Asaka, Jugloff, Zhang, Eubanks, & Fitzsimonds, 2006; Nelson, Kavalali, & Monteggia, 2006). Mice carrying a heterozygous mutation in the Dgrc8 gene, which is one of the genes disrupted by the 22q11.2 microdeletions and is a risk factor for schizophrenia, also display increased short-term depression (Fénelon et al., 2011a). Mice with a disruption in DISC1, a risk factor for schizophrenia, display reduced paired-pulse facilitation and enhanced short-term depression in the prefrontal cortex (Crabtree et al., 2017). This is likely due to widening of action potentials in layer 2/3 pyramidal neurons of the DISC1 mice (Crabtree et

al., 2017). The greater calcium entry increases the probability of release, which tends to switch the synaptic dynamics from facilitation to depression.

These findings suggest that altered short-term synaptic plasticity may contribute to impaired social cognition in neurodevelopmental disorders. This idea also receives support from a study investigating the electrophysiological effects of early weaning in mice. At the behavioural level, early weaning of mice leads to enhanced anxiety and impaired social interaction. Electrophysiological investigations reveal that this phenotype is accompanied by a reduction in paired-pulse facilitation in the prefrontal-amygdala pathway (Takita & Kikusui, 2016).

3.1.10 Neurexins, NRXN1α and synaptic function

Neurexins are a family of three genes (in humans: NRXN1, NRXN2 and NRXN3) that encode pre-synaptic cell-adhesion proteins that play a role in synapse formation and function. The NRXN1 gene encodes two protein isoforms, a long neurexin1-α protein and a shorter neurexin1-β protein. The promoter for neurexin1-α is upstream of exon 1 whereas the promoter for neurexin1-β is located in the intron downstream of exon 17 of the NRXN1 gene. Therefore, it is possible for mutations in the NRXN1 gene to block expression of neurexin1-α, but without affecting expression of neurexin1β-. Microdeletions in the NRXN1α gene are risk factors for multiple neurodevelopmental disorders (Kim et al., 2008; Kirov et al., 2009; Dabell et al., 2013; Yangngam et al., 2014; Agha et al., 2015).

Multiple lines of evidence suggest that NRXN1-α plays a role in regulating synaptic function, and in particular, in pre-synaptic vesicle release. Neurexin1-α is a receptor for the neurotoxin alpha-latroxin, which triggers neurotransmitter release at the pre-synaptic terminal. Neurexin 1 also binds to multiple molecules involved in pre-synaptic vesicle release, including synaptotagmin, CASK, Mints and Munc18, MUPP1 and syntenin, as well as post-synaptic

molecules, including neuroligins and LRRTMs (Biederer & Südhof, 2000; Hata, Butz, & Südhof, 1996; Hata, Davletov, Petrenko, Jahn, & Südhof, 1993; Jang et al., 2014; Ko, Fuccillo, Malenka, & Südhof, 2009). The fact that neurexin 1 binds to molecules involved in vesicle release suggests that it may help regulate vesicle release. The interaction with synaptotagmins particularly supports this claim, since syntaxin, a component of the SNARE complex, also appears to bind to this complex (O'Connor, Shamotienko, Grishin, & Betz, 1993). In addition, the drosophila homolog of alpha-NRX binds to N-ethylmaleimide-sensitive factor enzyme, which plays a role in SNARE disassembly.

Electrophysiological studies also support a role in pre-synaptic function. Loss of neurexins does not appear to affect excitatory synapse number (Etherton et al., 2009a; Missler, Zhang, Rohlmann, Kattenstroth, Hammer, Gottmann, & Sudhof, 2003). However, in mice, double or triple KO of *Nrxn1α, 2α,* and *3α* leads to a reduction in mEPSP frequency (Missler, Zhang, Rohlmann, Kattenstroth, Hammer, Gottmann, & Südhof, 2003). In addition, *Nrxn1α* KO mice have been reported to show a reduction in mEPSP frequency in the hippocampus (Etherton et al., 2009).

Nrxn1α has been implicated in the short-term synaptic dynamics evoked by repetitive stimulation. Neurexin-alpha double and triple KO mice display reduced paired-pulse facilitation (Missler, Zhang, Rohlmann, Kattenstroth, Hammer, Gottmann, & Südhof, 2003). A study investigating rats carrying the *Nrxn1α* KO deletion observed a reduction in paired-pulse facilitation in the amygdala (Twining et al., 2017). In contrast, in a later study, no changes in paired-pulse ratio were seen in Nrxn1α KO mice in the hippocampus (Etherton et al., 2009a), despite reduced mEPSP frequency. In drosophila, Nrx mutant synapses display increased short-term depression (Li et al., 2015).

Mice carrying a homozygous microdeletion in *Nrxn1α* display impairments in social behaviour, including increased aggression and heightened social approach (Grayton et al., 2013). My results from chapter 2 indicate that these deficits are persistent and occur in the absence of deficits in non-social cognition, suggesting that these mice may provide a good model for studying social dysfunction. In particular, the deficits in social behaviour observed in *Nrxn1α* KO rodents suggest that they possess an impaired regulation of aggression, disinhibition, and deficits in the ability to interpret social information. Indeed, *Nrxn1α* KO mice display aggression in "inappropriate" contexts (e.g. during interaction with juvenile mice). Moreover, *Nrxn1α* KO rats display impaired social fear learning (Twining et al 2017).

Aggression and disinhibition are common symptoms of orbitofrontal damage (see section 1.1.2.). Hence, orbitofrontal dysfunction may contribute to the social cognitive impairments in *Nrxn1α* KO mice. However, the orbitofrontal cortex has not yet been examined in *Nrxn1α* KO mice. In addition, no studies have yet examined whether alterations in synaptic function are pathway-specific and vary according to whether connections are local or long-distance.

The aim of this chapter was to investigate layer 2/3 excitatory circuitry in the orbitofrontal cortex of *Nrxn1α* KO mice. In particular, I explored the hypothesis that neurodevelopmental disorders are characterised by imbalances in long-range and short-range connectivity by examining excitatory post-synaptic potentials evoked in response to local stimulation, and stimulation of the prelimbic cortex.

3.2 Methods

3.2.1 Neurexin1α mice

Nrxn1α mice were generated and maintained as described in Chapter 2. Male and female *Nrxn1α* KO and WT mice aged between 10-20 weeks were investigated in this study.

3.2.2 Brain Slice Preparation

Mice were deeply anaesthetised with 3% Isoflurane (Maerial Animal Health Ltd., Harlow, UK) in 1 L,min^{-1} O_2. I ensured that pain reflexes were abolished by pinching the hind limb firmly. The mouse brain was then cooled rapidly by transcardiac perfusion of ice-cold dissection artificial cerebrospinal fluid (aCSF). I did this by cutting the skin in the midline from the lower abdomen to the neck. The xiphisternum was clamped and the right and left internal jugular neck veins were cut. I cut the ribs laterally and raised the rib cage to expose the heart. I inserted a needle into the left ventricle and cold dissection artificial cerebrospinal fluid (aCSF) was pumped into the heart for 2-3 minutes. The dissection aCSF contained: (in mM): 108 choline chloride, 3 KCl, 26 NaHCO3, 1.25 NaH2PO4, 25 Dextrose, 3 Na pyruvate, 2 CaCl2, 1 MgCl2, 285 mOsm, bubbled with 95%O_2/CO_2. Following transcardiac perfusion, the brain was dissected out and placed in ice-cold dissection aCSF, which had an osmolality between 285-295 mOsm. I put the mouse brain on a block, which was then submerged in a chamber filled with dissection aCSF. I cut coronal slices 300µm thick with a vibratome (Camden Instruments Ltd, Loughborough, UK) through medial prefrontal cortex (mPFC) starting from the frontal pole until the genu of the corpus callosum was visible to the naked eye.

I transferred the brain slices to a holding chamber in a water bath containing aCSF heated to 37°C. Slices were kept at 37°C for one hour and then maintained at room temperature. Slices were given at least one and a half hours to recover before being transferred to the recording chamber. The aCSF in the holding chamber contained (in mM): 124 NaCl, 2.3 KCl, 26 NaHCO3, 1.43 KH2PO4, 10 Dextrose, 1 MgSO4, and 2 CaCl2. Osmolality of the aCSF was monitored and maintained at 285-295 mOsm throughout the experiment.

3.2.3 Electrophysiological Recordings

Slices were placed in a recording bath containing artificial cerebrospinal fluid circulating at a speed of 3.5 ml/min. Slices were stabilised using a 'grating' consisting of an arc of flattened platinum wire crossed by strands of dental floss. The aCSF was kept at physiological temperature (37°) and bubbled with 95%O_2/5%CO_2. Slices were allowed a minimum of 15 minutes to warm up from room temperature before I started my recordings so that the input resistance of neurons restabilised at more physiological values. Brain slices were first viewed under bright field transillumination using 2x and 5x magnification (Olympus UK Ltd., Southall, UK). Patch clamp recordings were made from imaged neurons. To do this, I used infrared differential interference contrast microscopy (Olympus, UK Ltd., Southall, UK) with a 40x, 0.8 NA water immersion objective and a charge-coupled device camera (SPOT Insight QE; Diagnostic Instruments Inc., Sterling Heights, MI).

I recorded neurons from the ventrolateral and lateral orbitofrontal cortex. Single neurons were patched with pipettes with a resistance between 4-8 MΩ pulled from filamented borosilicate glass capillaries (1.5 mm outer diameter, 0.86 mm inner diameter; Harvard Apparatus Ltd., Edenbridge, UK) using a Flaming/Brown micropipette puller (Model P-97; Sutter Insrtument Co., Novato, CA). Pipettes were filled with an internal solution consisting of (in mM): 130 KMeSO4, 8 NaCl, 2 KH2PO4, 2 Dextrose, 10 HEPES, 1 Alexa Fluor 568, 285 mOsm, pH 7.30 at room temperature.

Cells were approached in voltage-clamp mode to form a gigaseal (cell attached resistance ≥ 1 GΩ). Once the gigaseal had been formed, I set the holding voltage to – 70 mV and ruptured the patch to enter the whole-cell recording configuration. I then switched from voltage clamp recording to current clamp recording mode. All recordings were made with an Axon Multiclamp 700A amplifier (Molecular Devices Corporation, Sunnyvale, CA), which was controlled digitally by Multiclamp Commander software. Pipette capacitance was

compensated (4-7 pF) and bridge balance was adjusted to remove the effect on my recordings of the pipette access resistance (10-30 MΩ). I monitored the pipette capacitance and access resistance during my recording and adjusted their compensation as necessary to optimize the quality of my recordings. Neurons with an access resistance above 40 MΩ or that increased by more than 20% during the recording epoch were discarded. Action potentials were filtered at 10 kHz and miniature post-synaptic potentials were filtered at 3 kHz using a low-pass 8-pole Bessel filter. Recordings were digitised at 20 k Samples by a PCIe 6321 DAQ card (National Instruments, Austin, TX, USA) in a rig computer and stored on its hard drive. Data acquisition was controlled by custom written software by Dr Gerald Finnerty in Labview (National Instruments, Austin, TX, USA).

Neurons with a resting membrane potential more depolarized than -65 mV were discarded. The identity of pyramidal neurons was confirmed electrophysiologically by injection of suprathreshold, depolarising current pulses lasting 500 ms. Pyramidal neurons were required to display: a regular-spiking pattern of action potentials; and action potential peak amplitudes above +30 mV.

3.2.4 Region identification and position of recorded neurons in brain slices

Neurons from layers 2 and 3 of the ventrolateral and lateral orbitofrontal cortex were recorded for these studies (Fig. 17A). Regions were identified with the help of the Allen Brain Atlas (Fig. 17B). Neurons were recorded in the region starting at the most medial and ventral portion of the frontal cortex midline. The distance of the neurons from the pia was calculated to determine the layer in which the neuron was recorded with assistance from the Allen Brain Atlas.

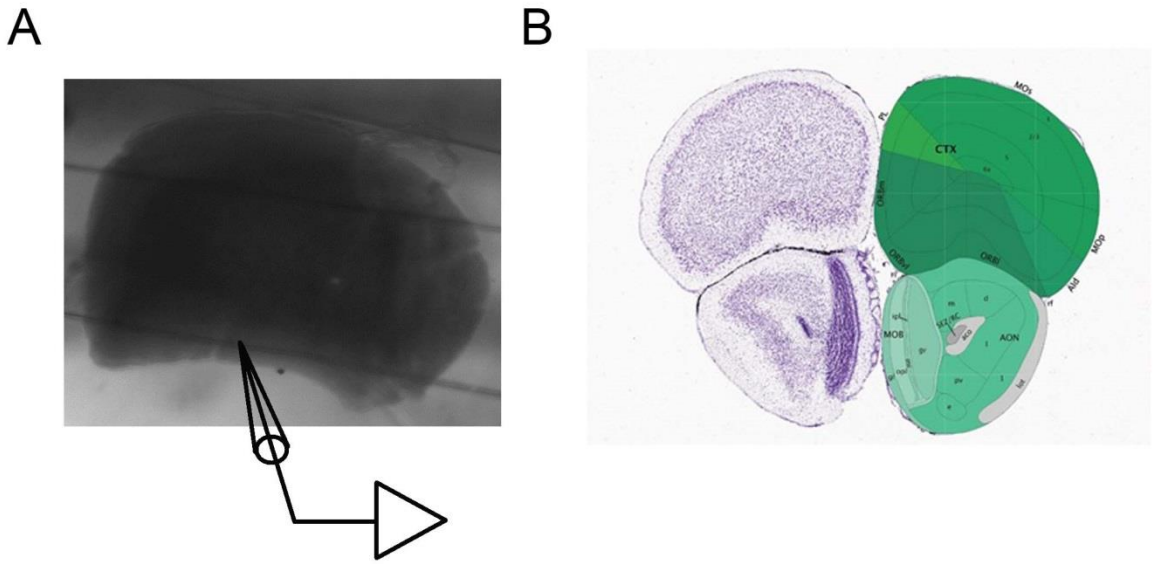

Figure 17: Mouse orbitofrontal cortex.

A: Example image of a mouse orbitofrontal cortex slice, with a depiction of a pipette through ventrolateral orbitofrontal cortex. B: Allen brain atlas coronal section through the frontal lobe with orbitofrontal cortex above the olfactory bulb.

3.2.5 Passive membrane properties

A -0.1 nA, 100 ms duration hyperpolarising current pulse was injected into the neuron within 5 minutes of achieving the whole-cell recording configuration. This current pulse was used to measure neuronal input resistance, and membrane time constant. Neuronal input resistance was monitored during recording to ensure that neurons remained healthy throughout the recording epoch. In a healthy neuron, input resistance remains stable over recording, except for a small increase after break-in.

3.2.6 Recording of action potential properties and excitability

Action potential properties and excitability were assessed by recording the responses of neurons to injection of 500 ms duration depolarising current pulses, usually ranging from 0.2 – 1.0 nA in 0.2 nA increments. Other amplitude current injections were sometimes tested. In addition, the minimum amplitude of injected current to elicit a single action potential on half

of the traces was determined. This is termed the rheobase. The number of neurons per group is displayed in Table 1.

Table 1: Number of neurons in *Nrxn1α* WT and KO male and female groups for which intrinsic excitability was recorded.

	WT	KO
Male	10	18
Female	5	14
Total	15	32

3.2.7 Recording of spontaneous and miniature activity

I recorded neural activity in the absence of stimulation, which I refer to as spontaneous activity, immediately after collecting excitability data (500 ms – 5s traces). I then washed tetrodotoxin citrate (1 µM) and picrotoxin (100 µM) into the bath to record miniature excitatory post-synaptic potential (mEPSPs) activity. 1.0 nA and a 0.4 nA, 500 ms duration depolarising current pulse were applied every 2 minutes to identify when action potential firing was fully blocked. I found that action potentials were abolished 8 minutes after the drugs were added to the circulating aCSF. mEPSPs were recorded 12-15 minutes after wash-in with traces of 5 s duration of spontaneous activity. One neuron was recorded per slice. I recorded from a maximum of four slices per animal to avoid biasing the data (Table 2).

Table 2: Number of neurons in *Nrxn1α* WT and KO male and female groups for which mEPSPs were recorded.

	WT	KO
Male	8	17
Female	5	14
Total	13	31

Distance from the pia was recorded. Mean distance ± sem from the pia was 325 ± 17 μm (WT: 295 ± 27 μm, KO: 338 ± 22 μm).

3.2.8 Recording of evoked excitatory post-synaptic potentials in response to stimulation

Evoked EPSP activity was collected after wash-on of 50 μM picrotoxin. This was achieved 8 minutes after the drugs were added to the aCSF. A train of eight pulses was delivered at a frequency of 10, 20 and 40 Hz. EPSP amplitude, shape and dynamics were measured a minimal stimulation protocol (Finnerty, Roberts, & Connors, 1999). Minimal stimulation was defined as the current injection required to evoke an EPSP on each pulse of the train reliably on 50% of trials.

Axons were stimulated with extracellular current pulses generated by a DS2 constant current stimulator (Digitimer, Welwyn Garden City, Herts, UK) and applied through bipolar concentric fine-tip electrodes (FHC, Bowdoin, Maine, USA). Local stimulation was achieved by stimulating axons (mean distance from post-synaptic cell: 460 ± 31 μm; WT: 366 ± 66

μm, KO: 554 ± 47 ± μm) in layer 2/3 ventral orbitofrontal cortex with pulses of a duration of 50 μs and a current amplitude ranging from 10-60 μA (Fig. 18). Stimulation of longer-distance axons was achieved by stimulating axons in layer 2/3 prelimbic cortex (mean distance from post-synaptic cell: 968 ± 56 μm, WT: 949 ± 54 μm, KO: 988 ± 75 μm) with pulses of a duration of 100 μs and a current amplitude ranging from 40-200 μA (Table 3).

Table 3: Number of neurons in *Nrxn1α* WT and KO male and female groups for which mEPSPs were recorded.

	Local Pathway		**Long-range Pathway**	
	WT	**KO**	**WT**	**KO**
Male	7	17	3	5
Female	13	9	7	6
Total	20	26	10	11

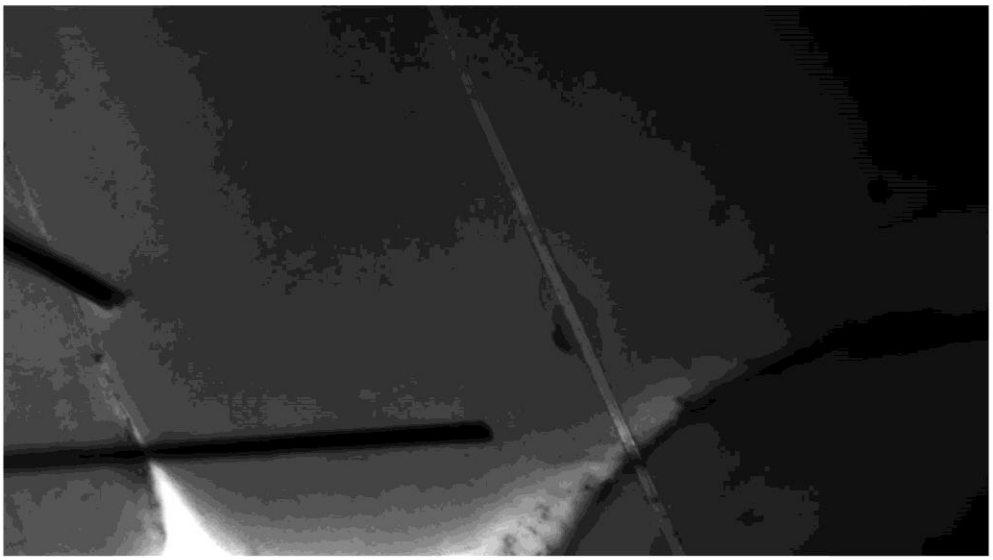

Figure 18: Electrode position in orbitofrontal cortex.

DIC Image of mouse orbitofrontal cortex with stimulating electrodes positioned in layer 2/3 ventrolateral orbitofrontal cortex (local stimulation pathway) and in layer 2/3 prelimbic cortex (long-range stimulation pathway).

3.2.9 Electrophysiological analysis

Analysis was conducted using custom-written VIs (written by Gerald Finnerty) in Labview 14.0 unless otherwise noted.

Passive membrane properties

Input resistance (Rm), membrane capacitance (Cm) and membrane time constant (τ) of neurons were determined from responses to injection of -0.1 nA 100 ms duration hyperpolarising square pulses. Values were measured using the Labview VI 'GTF Passive membrane properties'. Rm was calculated using Ohm's Law, with voltage change defined as

the change in membrane potential from baseline to maximum hyperpolarisation. To determine the membrane time constant, an exponential curve was fitted to the membrane potential hyperpolarisation to calculate the time taken to reach the fraction (1 – 1/e) of maximal hyperpolarisation. Cm was determined using the relationship, membrane time constant = Rm x Cm. Passive membrane properties were compared across *Nrxn1α* WT and KO male and female mice. In addition, the resting membrane potential of the neurons was compared across groups, which was measured 5 minutes after break-in.

Excitability

The number of action potentials generated by a 500 ms duration depolarising current pulse was counted using the Labview VI 'GTF AP detector 1.7'. This was counted for pulses varying between 0.2 – 1.0 nA. The relationship between current injection and action potential number is roughly linear within this range (Cheetham, Hammond, Edwards, & Finnerty, 2007)). Hence, a linear regression of the relationship between current injection and number of action potentials was performed for each neuron. The slopes of the linear regression lines were compared across groups. In addition, the current required to generate one action potential (threshold) was calculated using the regression line equation $y = mx + C$.

Miniature excitatory post-synaptic potential activity

Two five second traces of spontaneous activity were analysed per neuron. Analysis was performed manually using the Labview VI 'GTF spontaneous EPSPs manual'. mEPSPs were defined using the shape criteria described in our lab previously: a depolarising current with a time-to-peak < 5ms, a rate of decay after peak that was between 2% of peak mEPSP amplitude per ms and (50/time to peak)% of peak mEPSP amplitude per ms (Cheetham et al., 2007). In addition, mEPSPs were required to have an amplitude ≥ 87.5 μV. This threshold was determined by calculating 2.5 times the root mean square of the noise across traces.

EPSPs that overlapped (collided) were considered to be separate when the second EPSP started > 1 ms after the peak of the first EPSP. The amplitude of mEPSPs was measured by positioning vertical cursors at a baseline region and at the EPSP peak. The vertical cursors measure the average amplitude within a 1 ms time window around the position of the cursor. In addition, the number of mEPSPs per trace was counted to determine mEPSP frequency. mEPSP amplitude and frequency were compared across conditions.

Evoked excitatory post-synaptic potential activity

EPSPs were evoked by a train of 8 pulses delivered at a current injection intended to evoke minimal stimulation at 10, 20 and 40 Hz. EPSP measurements were made after averaging EPSP amplitudes across 10 trials. The time between trials was 10 seconds. The following criteria were used to differentiate evoked EPSPs from spontaneous activity:

Trials in which there was excessive spontaneous activity, epileptic activity or polysynaptic activity were discarded from analysis. Traces were discarded from analysis if there was no clear EPSP 1 as this suggested that my minimal stimulation protocol did not reliably evoke a baseline response.

The baseline EPSP amplitude (first response in the train, EPSP1) was calculated by measuring the EPSP amplitude of the first pulse in a train of 8 pulses and averaging amplitude over the 10 Hz, 20 Hz and 40 Hz conditions.

Facilitation was measured by examining the first 100 ms after the first response in the train (EPSP1), since neurons displayed the greatest facilitation during this time. The degree of facilitation was calculated by averaging the amplitude of the EPSPs occurring in the first 100 ms after EPSP1 and dividing this averaged amplitude by the amplitude of the EPSP evoked on pulse 1. The steady-state ratio was calculated by averaging the EPSP amplitudes of the last

three EPSPs in the train (EPSP5 – 8) and dividing this by the amplitude of the EPSP evoked on pulse 1 (EPSP1).

3.2.10 Statistical analysis

Statistical tests were performed in SPSS or Sigmaplot unless otherwise stated. For data that passed the Kolmogorov-Smirnov test for normality and Levene's median test for equal variance, mean and standard error of the mean were calculated, and data were analysed using independent samples t-tests or mixed ANOVA as appropriate. Log transforms were applied on non-normally distributed data. If this did not resolve violations of normality and equal variance, median and interquartile range was calculated and non-parametric statistics were employed. For analysis of short-term dynamics, the distance of the cell from the stimulating electrode and sex of the animal were covaried out using ANCOVA.

3.3 Results

3.3.1 Passive Membrane Properties

Passive membrane electrical properties of a cell can affect the way in which the cell responds to synaptic inputs, and hence, the propensity of the cell to fire action potentials. As such, I first sought to determine whether the passive membrane properties of pyramidal cells were altered in *Nrxn1α* KO mice.

Resting membrane potential was measured a few minutes after breaking into the cell. I found no significant differences in resting membrane potential between WT and *Nrxn1α* KO pyramidal cells in layer 2/3 of orbitofrontal cortex (WT, Vm = -73 ± 1 mV, n = 15 neurons; KO, Vm = -73 ± 1 mV n = 32 neurons; $t(49) = -0.324$, $p = 0.747$) (Fig. 19A).

I found no difference in membrane resistance of layer 2/3 pyramidal neurons in orbitofrontal cortex of *Nrxn1α* mice compared with WT mice (WT, Rm =79 ± 9 MΩ, n = 15 neurons; KO Rm = 69 ± 7 MΩ, n = 32 neurons; t(49) = -0.785, p = 0.436) (Fig. 19B).

The membrane time constant (τ_m) reflects the time required to change the membrane potential by 1-(1/e) of the final change in voltage. I found that there was no difference in the membrane time constant of layer 2/3 pyramidal neurons in orbitofrontal cortex of WT and *Nrxn1α* KO mice (WT, τ_m = 9.7 [9.4-14.0] ms, n = 15 neurons; KO, τ_m = 9.9 [9.4-10.6] ms, n = 32 neurons; U = 294, p = 0.853, Mann-Whitney U test) (Fig. 19C).

Finally, the membrane capacitance (C_m) of pyramidal neurons was similar in WT and KO pyramidal cells recorded from layer 2/3 of orbitofrontal cortex (WT, C_m = 154 [138-232] pF, n = 15 neurons; KO, C_m = 172 [117-238] pF, n = 32 neurons; U = 276, p 0.592, Mann-Whitney U test) (Fig. 19D).

In summary, I found no difference in the passive membrane properties of layer 2/3 pyramidal neurons in orbitofrontal cortex of WT or *Nrxn1α* mice.

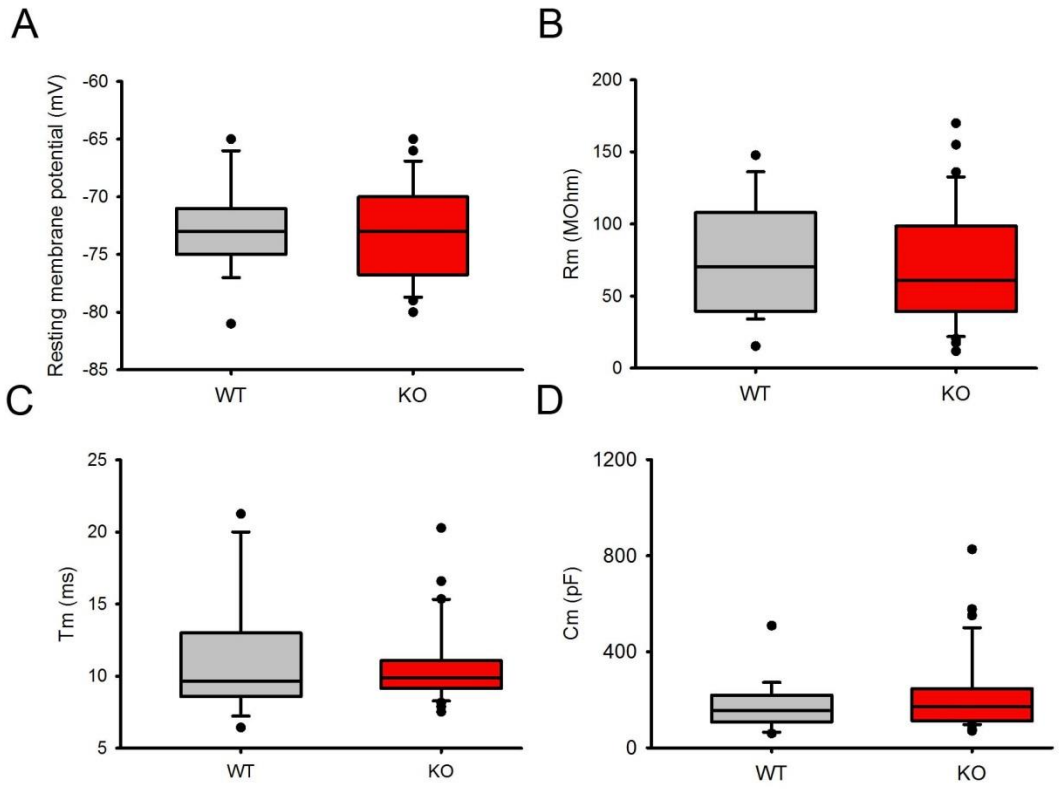

Figure 19: Passive membrane properties of layer 2/3 orbitofrontal pyramidal neurons in WT and Nrxn1α KO mice.

A: Box and whisker plot of resting membrane potential of layer 2/3 pyramidal neurons in orbitofrontal cortex of WT (grey) and *Nrxn1α* KO mice (red). B: Box and whisker plot of input resistance (MΩ) in WT (grey) and KO pyramidal neurons (red). C: Box and whisker plot of time constant (T_m) in WT (grey) and KO pyramidal neurons (red). D: Box and whisker plot of membrane capacitance (C_m) in WT (grey) and KO (red) pyramidal neurons.

3.3.2 Intrinsic Excitability and Action Potential Firing

Neurons were classified as pyramidal on the basis of their pyramidal shaped soma when imaged and electrophysiological properties, including input resistance and firing pattern. Orbitofrontal pyramidal neurons displayed regular-spiking neural firing (Fig. 20A).

The excitability of layer 2/3 excitatory pyramidal neurons in orbitofrontal cortex was measured by plotting action potentials firing changes with increasing current injections (100-1000 pA) to generate an input-output curve. Since these plots are roughly linear in the range of +100 pA to +1000 pA, a line was fitted to the data for each cell, as we have done previously in my laboratory (Cheetham et al., 2007). The line of best fit was used to determine the slope and rheobase of for each cell (Fig. 20B).

I found that the slope of the input-output function was not altered in KO mice compared with WT mice (WT: 0.036 ± 0.004 $Hz.pA^{-1}$, n = 15 neurons, KO; 0.040 ± 0.003 $Hz.pA^{-1}$, n = 32 neurons, $t(49) = 0.179$, $p = 0.858$). There was also no significant difference in the mean intercept in KO mice relative to WT mice (WT: -3.406 ± 1.247, n = 15 neurons, KO: -7.211 ± 1.369, n = 32 neurons $t(49) = -1.881$, $p = 0.066$) (Fig. 20C). The, rheobase was estimated from the input-output curve by calculating the current required to generate one action potential (y = 1) in the output-input function. There were no significant differences in estimated rheobase for layer 2/3 pyramidal neurons in orbitofrontal cortex of KO mice compared with WT mice (WT: rheobase = 87.10[1-199] mV, n = 15 neurons, KO: rheobase = 182.10[85-336] mV, n = 32 neurons, U = 209, $p = 0.066$, Mann-Whitney U Test) (Fig. 20D). I concluded that the *Nrxn1α* microdeletion did not affect the passive membrane properties or excitability of layer 2/3 pyramidal neurons in orbitofrontal cortex.

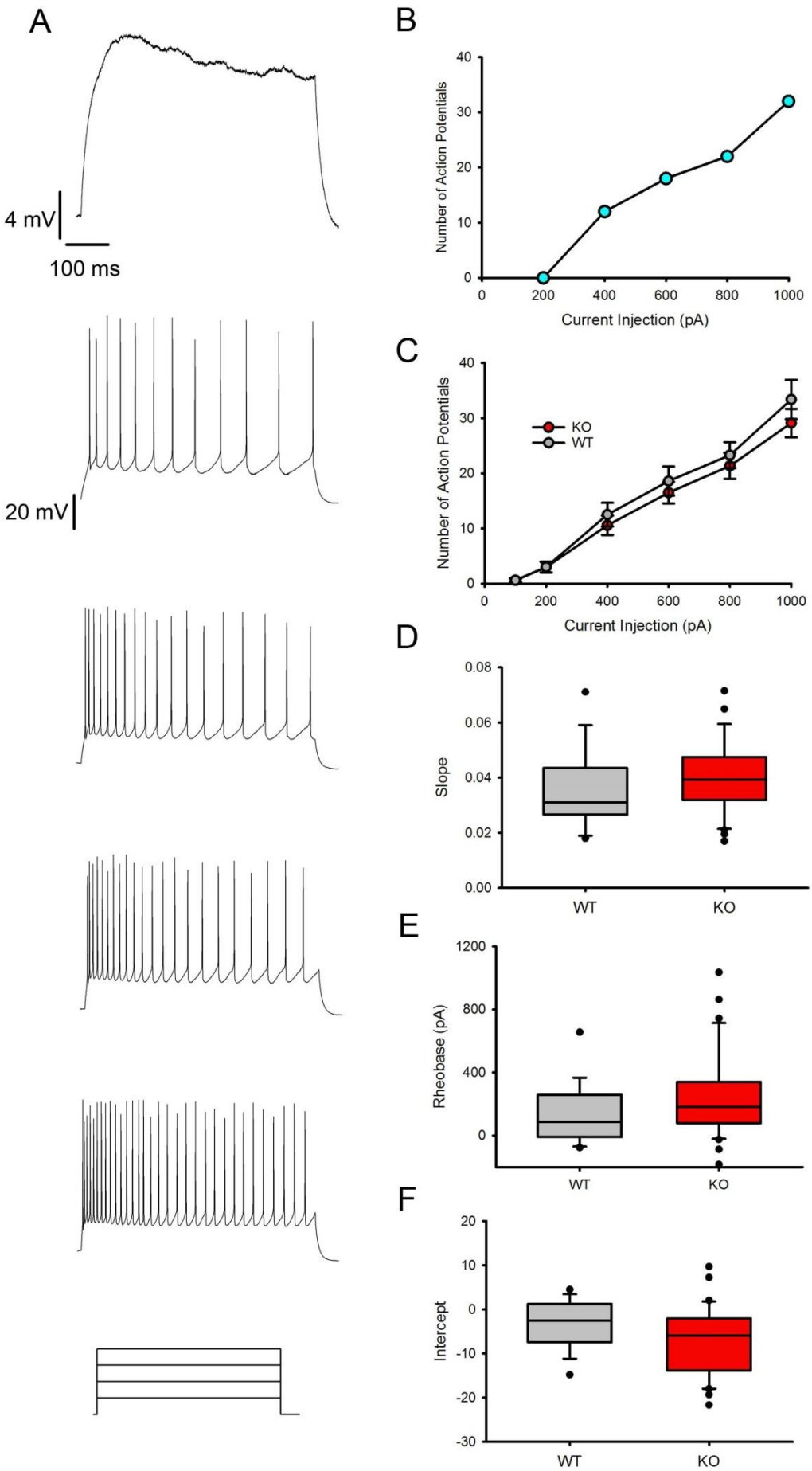
A
4 mV
100 ms
20 mV
B
Number of Action Potentials
Current Injection (pA)
C
KO
WT
Number of Action Potentials
Current Injection (pA)
D
Slope
WT
KO
E
Rheobase (pA)
WT
KO
F
Intercept
WT
KO

Figure 20: Intrinsic excitability of layer 2/3 pyramidal orbitofrontal neurons in WT and KO animals.

A: Example traces of action potentials in one layer 2/3 pyramidal neuron in orbitofrontal cortex evoked by 500 ms depolarising current pulses (200-1000 pA). B: Input-output function for a layer 2/3 pyramidal neuron in orbitofrontal cortex. C: Mean input-output functions ± sem in WT (grey) and KO neurons (red). D: Box and whisker plots of input-output function slope in WT and KO neurons. E: Box and whisker plots of extrapolated rheobase (threshold for firing of one action potential) in WT and KO neurons. F: Box and whisker plots of intercept in WT and KO neurons.

3.3.3 Mini Excitatory Post-synaptic Potentials (mEPSPs)

Miniature EPSPs (mEPSPs) are small EPSPs that occur spontaneously in the absence of action-potential generated activity (del Castillo & Katz, 1954; Fatt & Katz, 1952) (Fig. 21A). mEPSPs are thought to reflect release of a single vesicle at at one synapse. The frequency and amplitude of miniature EPSPs provides a measure of the strength of excitatory drive onto a post-synaptic neuron. Therefore, mEPSPs represent a good starting point for exploring disruptions in excitatory circuitry.

mEPSP frequency is predominantly pre-synaptically determined, and depends on both the number of synapses at a post-synaptic neuron, and the rate of action potential independent vesicle release at individual synapses. Neurexin-1α is a pre-synaptic protein that is thought to play a role in regulating synaptic function. The black widow spidertoxin, α-latrotoxin, induces release of neurotransmitter by binding to neurexin-1α at presynaptic terminals (Geppert et al., 1998). Hence, it is possible that knock-out deletion of *Nrxn1α* might alter mEPSP frequency. Indeed, in a previous study, *Nrxn1α* KO mice displayed reduced mEPSP frequency in the hippocampus, although this study was conducted with mice maintained on a mixed backrgound. In addition, triple and double neurexin KO mice display reduced mEPSP

frequency in the absence of changes in the number of synapses, suggesting a role in synaptic function.

As such, I sought to determine whether mEPSP frequency was altered in *Nrxn1α* KO mice. The mean frequency of mEPSPs was not significantly altered in *Nrxn1α* KO pyramidal neurons relative to WT pyramidal neurons (WT, 13 ± 1 Hz, n = 13, neurons; KO, 13 ± 1 Hz, n = 31, neurons; t(42) = 0.160, p = 0.873, t-test) (Fig. 21B, C).

Next, I sought to explore whether mEPSP amplitude was affected in *Nrxn1α* KO mice. Since mEPSPs reflect activity at a single synapse, the amplitude of mEPSPs is not influenced by synapse number. mEPSP amplitude is influenced by the density of AMPA and NMDA receptors at the post-synapse and the amount of neurotransmitter released. There was no significant difference in the mean amplitude of mEPSPs in *Nrxn1α* KO pyramidal neurons compared with WT pyramidal neurons (WT, 0.16 ± 0.01 mV, n = 13 neurons; KO, 0.16 ± 0.01 mV, n = 31 neurons, t(42) = 0.414, p = 0.681, t-test on log transformed data) (Fig. 21D, E).

Finally, I explored whether mEPSP amplitude and frequency were influenced by the cortical layer from which neurons were recorded. Neither mEPSP amplitude nor mEPSP frequency correlated with distance of the neurons from the orbitofrontal pia in either WT neurons (frequency: r = -0.221, n = 13 neurons, p = 0.488, amplitude: r = -0.144, n = 13, p = 0.448) or *Nrxn1α* KO neurons (frequency: r = -0.361, n = 31 neurons, p = 0.225, amplitude: r = 0.097, n = 31 neurons, p = 0.612).

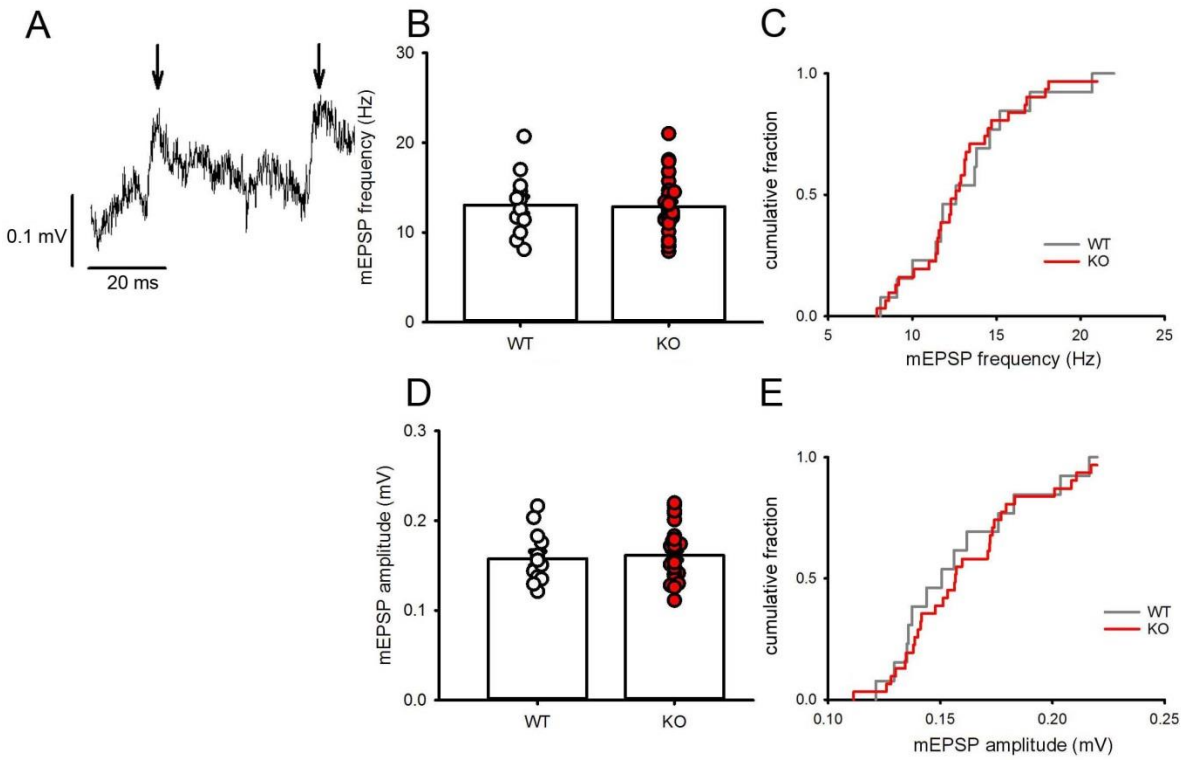

Figure 21: mEPSPs in WT and KO orbitofrontal pyramidal neurons.

A: Example trace (100 ms) of spontaneous mEPSP activity in an orbitofrontal pyramidal neuron. Arrows point to mEPSPs. B: Mean ± sem mEPSP frequency (Hz) in WT and KO neurons with overlaid scatter. C: Cumulative fraction plot displaying mEPSP frequency in WT and KO neurons. D: Mean ± sem mEPSP amplitude in WT and KO neurons with overlaid scatter of data from WT (empty circles) and *Nrxn1α* KO layer 2/3 pyramidal neurons (red).E: Cumulative fraction plot displaying mEPSP amplitude in WT and KO neurons.

In rodent somatosensory layers 2/3, mEPSC amplitude and frequency are altered in opposite directions during critical period plasticity (Desai, Cudmore, Nelson, & Turrigiano, 2002). However, in rodent orbitofrontal cortex, the relationship between mEPSP amplitude and frequency remains unexplored. As such, I explored whether mEPSP frequency and amplitude were correlated in WT and KO neurons. There was a significant positive correlation between mEPSP frequency and amplitude in both WT and KO neurons (WT, $r = 0.976$, $n = 14$ neurons, $p < 0.001$; KO: $r = 0.974$, $n = 31$, $p < 0.001$, Fig. 22). This demonstrated that in both

WT and KO mice, layer 2/3 neurons in orbitofrontal cortex that displayed larger mEPSP amplitudes also displayed higher mEPSP frequencies.

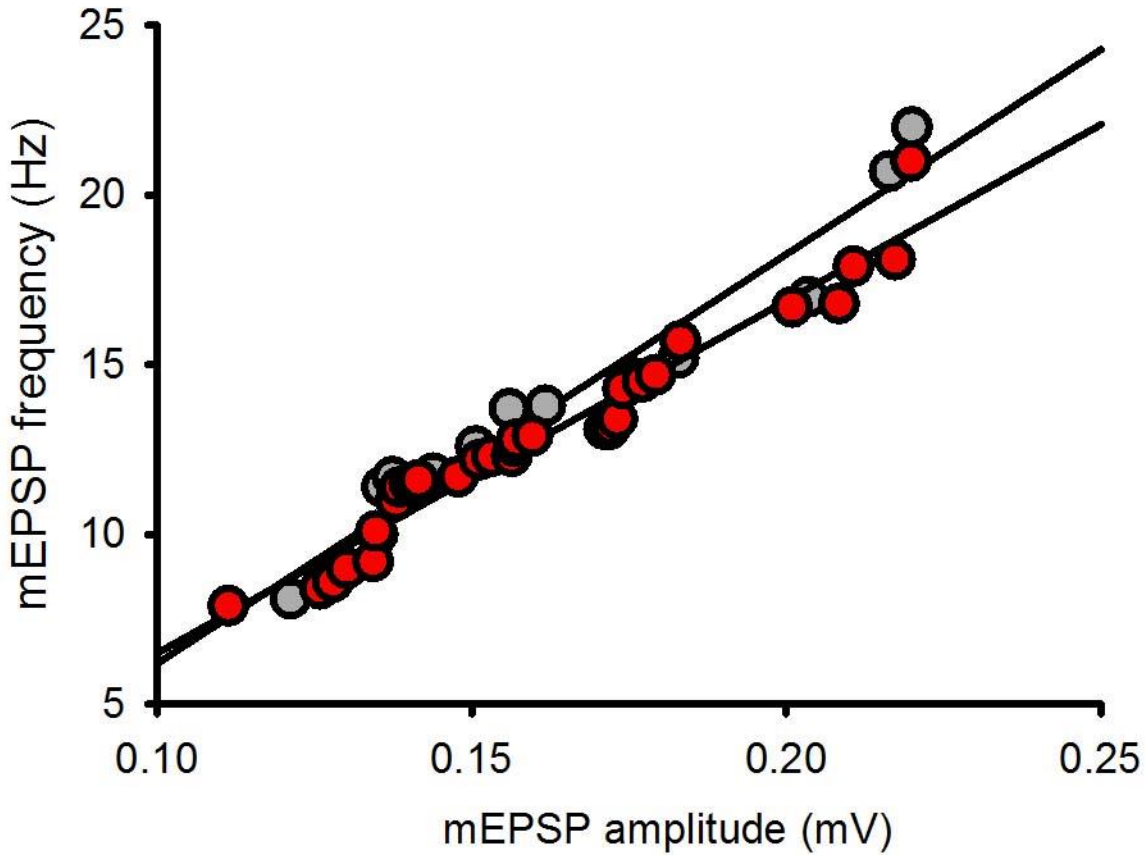

Figure 22: Correlation between mEPSP frequency and amplitude.

mEPSP amplitude and frequency are positively correlated in WT and KO pyramidal cells. Scatter plot of mEPSP amplitude versus mEPSP frequency in WT (grey, n = 13 neurons) and KO (red, n = 31 neurons) with linear regression lines.

In summary, I found no changes in excitatory circuitry in the orbitofrontal cortex of *Nrxn1α* KO mice via the investigation of mEPSPs. However, mEPSPs possess limitations in their ability to provide insight into excitatory synaptic transmission in circuits. First, mEPSPs reflect global input onto a post-synaptic neuron. Recorded mEPSPs at a post-synaptic neuron originate from multiple different inputs and there is no way to determine the source (e.g.

layer and region) of these inputs. In addition, some evidence suggests that mEPSPs may originate from a different vesicle pool to those used during evoked release (Kavalali, 2014). To the extent that spontaneous vesicle release originates from a different pool to evoked vesicle release, mEPSPs may provide limited insight into the properties of evoked release. Therefore, I sought to explore excitatory circuitry in more detail by investigating EPSPs evoked in response to axonal stimulation.

3.3.4 Local and long-distance evoked excitatory post-synaptic potentials

I first examined the amplitude of evoked excitatory post-synaptic potentials (EPSPs). The size of EPSP 1 was comparable across genotypes and across stimulation pathway (WT: Vm = 0.35 ± 0.04 mV, n = 30 neurons, KO: Vm = 0.55 ± 0.09 mV, n = 37 neurons, genotype factor: $F(1,63) = 2.683$, $\eta^2 = 0.041$, $p = 0.106$, pathway factor: $F(1,63) = 0.095$, $\eta^2 = 0.002$, $p = 0.759$, genotype x pathway interaction: $F(1,63) = 0.420$, $\eta^2 = 0.007$, $p = 0.519$, Fig. 23).

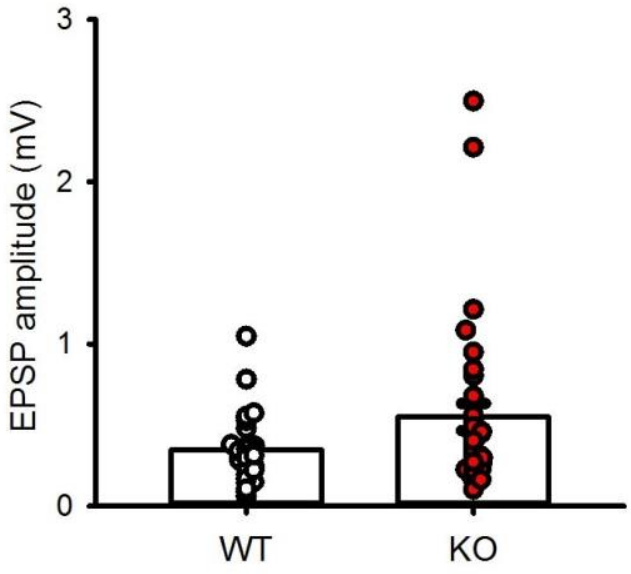

Figure 23: EPSP amplitude in WT and KO neurons.

Evoked EPSP amplitude (mV) in layer 2/3 orbitofrontal pyramidal WT (empty circles, n = 30 neurons) and KO (red, n = 37 neurons) neurons.

Next, I examined the short-term dynamics of EPSPs upon repetitive stimulation. The amplitude of EPSPs was normalised to the first EPSP in the train. Distance of the cell from the electrode and sex were covaried out. The majority of orbitofrontal layer 2/3 pyramidal neurons displayed initial facilitation which was most apparent 100 ms into the train. After initial facilitation, EPSP responses then declined to a steady-state (Fig. 24A, B). This facilitation appeared more prominent in the short-range pathway (Fig. 24B). Since the amplitude of EPSP1 was comparable across pathways and genotypes, responses were normalised to the amplitude of EPSP1 (Fig. 24Ci-iv).

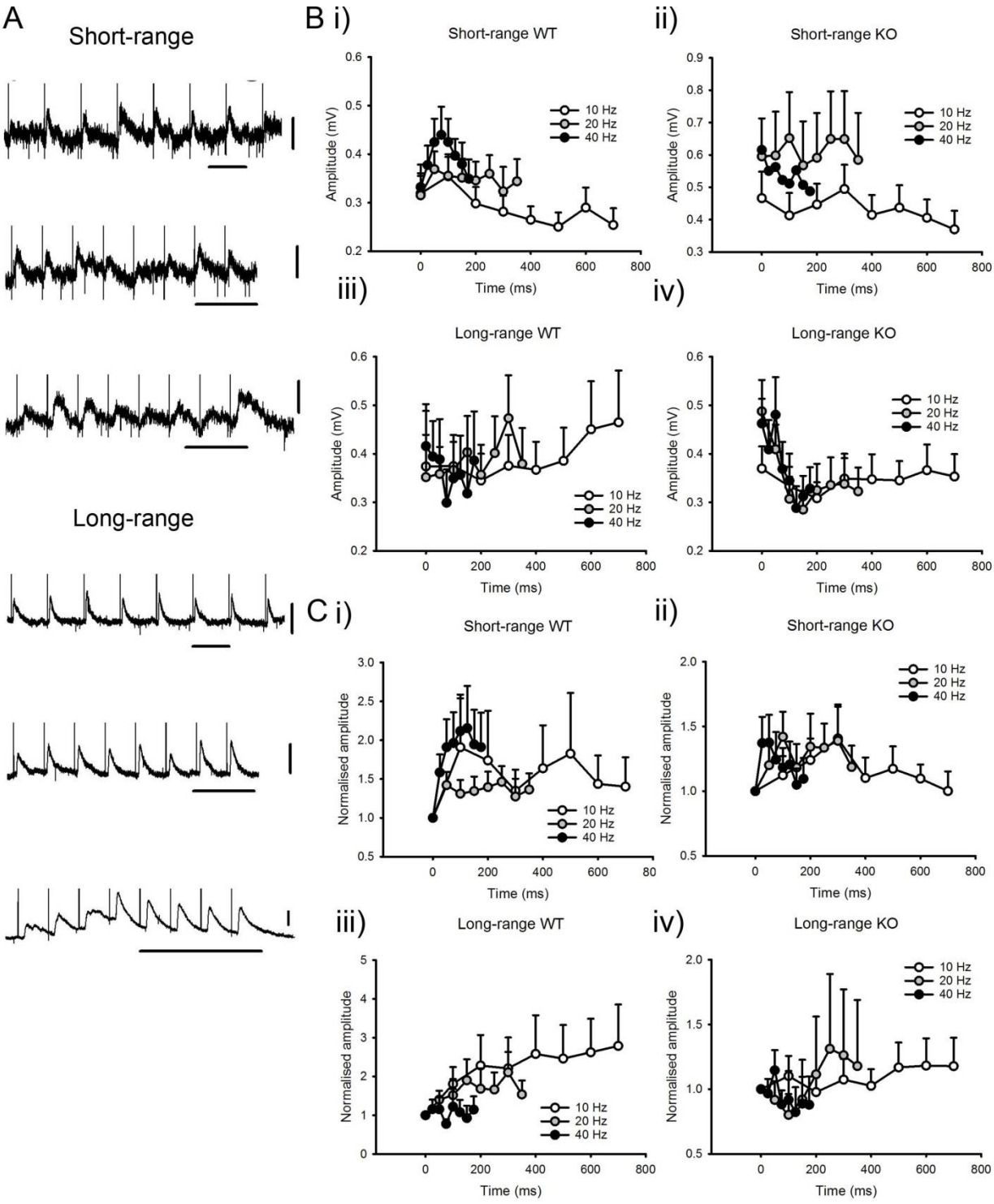
A
Short-range
Long-range
B i)
Short-range WT
ii)
Short-range KO
iii)
Long-range WT
iv)
Long-range KO
C i)
Short-range WT
ii)
Short-range KO
iii)
Long-range WT
iv)
Long-range KO
Amplitude (mV)
Normalised amplitude
Time (ms)
10 Hz
20 Hz
40 Hz

Figure 24:Short-term dynamics of EPSPs in orbitofrontal pyramidal layer 2/3 neurons evoked by local and long-range stimulation.

A: Example trace of evoked EPSP activity in an orbitofrontal pyramidal layer 2/3 neuron at 10, 20 and 40 Hz stimulation frequencies in local (short-range) and long-range pathways. B: Raw amplitude of evoked EPSPs plotted against time in trial in the short-range pathway in WT (i) and KO (ii) orbitofrontal layer pyramidal 2/3 neurons (i), and raw EPSP amplitude in the long-range pathway in WT (iii) and KO (iv) orbitofrontal layer pyramidal 2/3 neurons. C: Normalised EPSP amplitude plotted against time in trial (ms) in the short-range pathway in WT (i) and KO (ii) orbitofrontal layer pyramidal 2/3 neurons, and normalised EPSP amplitude in the long-range pathway in WT (iii) and KO (iv) orbitofrontal layer 2/3 pyramidal neurons.

I first sought to compare the degree of facilitation in orbitofrontal layer 2/3 pyramidal neurons. Since facilitation was most prominent in the first 100 ms, facilitation was measured by averaging the pulses occurring in the first 100 ms after pulse 1 and dividing this by pulse 1. WT orbitofrontal layer 2/3 pyramidal neurons displayed significantly greater facilitation than KO orbitofrontal layer 2/3 pyramidal neurons (WT: 1.5 ± 0.2; n = 30 neurons, KO: 1.0 ± 0.2, n = 37 neurons, $F(1,59) = 4.780$, $\eta^2 = 0.075$, $p = 0.033$). Facilitation was more apparent in the short-range pathway, although this did not reach significance ($F1,59) = 2.978$, $\eta^2 = 0.048$, $p = 0.090$, Fig. 25A).

I then explored the steady-state amplitude reached in orbitofrontal pyramidal layer 2/3 neurons. The steady-state amplitude responses were also significantly greater in WT than KO orbitofrontal pyramidal layer 2/3 neurons (WT: 1.6 ± 0.2, n = 30 neurons, KO: 1.0 ± 0.2, n = 37 neurons, $F(1,59) = 4.938$, $\eta^2 = 0.077$, $p = 0.030$, Fig. 25B). This finding was observed across all frequencies and in both the short and long-range pathway.

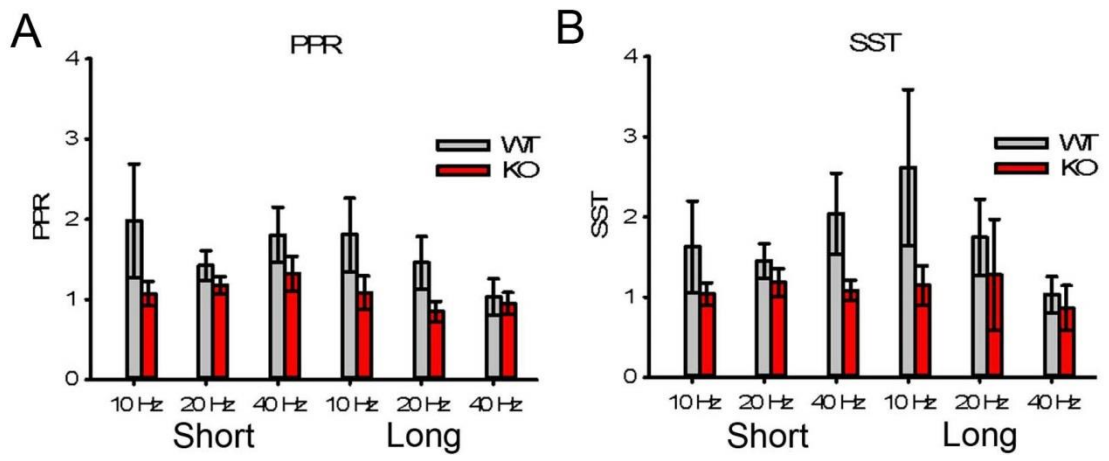

Figure 25: Paired-pulse and steady-state ratio of evoked EPSPs in orbitofrontal pyramidal layer 2/3 neurons in local and long-range pathways.

A: Bar chart depicting mean ± sem paired-pulse ratio (PPR) in the short and long-range pathway at 10, 20, and 40 Hz frequency stimulation in WT (grey) and KO (red) orbitofrontal layer 2/3 pyramidal neurons. B: Bar chart depicting mean ± sem normalised steady-state response (SSR) in the short and long-range pathway at 10, 20, and 40 Hz frequency stimulation in WT (grey) and KO (red) orbitofrontal layer 2/3 pyramidal neurons.

In summary, my results demonstrated that orbitofrontal layer 2/3 pyramidal neurons display reduced short-term facilitation in response to stimulation from both local connections within orbitofrontal cortex, and long-range pathways from prelimbic cortex.

3.4 Discussion

The aim of this chapter was to investigate orbitofrontal cortex circuitry in *Nrxn1α* KO mice. This mouse line carries a microdeletion which is a risk factor for multiple neurodevelopmental disorders, and displays selective deficits in social behaviour that may involve the orbitofrontal cortex. A main aim of the study was to explore whether *Nrxn1α* KO mice possess altered neural circuitry in the orbitofrontal cortex. I used recordings of mEPSPs to give a global measure of synaptic input onto layer 2/3 pyramidal neurons in orbitofrontal

cortex. I sought to compare local and long-range connectivity in the orbitofrontal cortex to examine the properties of these connections in the orbitofrontal cortex and determine whether *Nrxn1α* KO mice display pathway-specific changes in synaptic function. Finally, I also investigated whether intrinsic excitability was altered in *Nrxn1α* KO mice.

I found that:

- Local excitatory connections in the orbitofrontal cortex displays greater short-term facilitation than long-range excitatory connections from prelimbic cortex to the orbitofrontal cortex
- *Nrxn1α* KO mice display reduced short-term facilitation. This manifests as a shift towards short-term depression in the orbitofrontal cortex and is most obvious in local excitatory connections
- Deletion of *Nrxn1α* does not affect mEPSP frequency or amplitude
- *Nrxn1α* KO mice do not show altered intrinsic excitability

Multiple researchers have proposed that disorders of neurodevelopment such as autism and schizophrenia are accompanied by changes in synapse density (Courchesne & Pierce, 2005). Specifically, deficits in synaptic pruning have been proposed as a cause of neurodevelopmental disorders (Feinberg, 1982). This is consistent with neuropathology studies reporting changes in the number of dendritic spines in autism and in schizophrenia (Glantz & Lewis, 2000; Hutsler & Zhang, 2010; Irwin et al., 2001). A number of these studies implicate prefrontal cortical layers 2 and 3.

I found no changes in the intrinsic excitability and passive membrane properties of *Nrxn1α* KO orbitofrontal layer 2/3 pyramidal neurons. Given that neurexin1-α is a synaptic protein, changes in intrinsic excitability might not be expected. Nonetheless, alterations in synaptic

circuitry may lead to homeostatic changes in intrinsic excitability to compensate for the synaptic alterations. The absence of changes in intrinsic excitability in *Nrxn1α* KO neurons in this study is consistent with previous research (Etherton et al., 2009; Missler, Zhang, Rohlmann, Kattenstroth, Hammer, Gottmann, & Südhof, 2003; Twining et al., 2017), and with the fact that neurexin1-α is a synaptic protein.

I also found no changes in mEPSP frequency or amplitude in *Nrxn1α* KO mice. This contrasts with Etherthon and colleagues 2009, who observed a reduction in mEPSP frequency in the hippocampus of *Nrxn1α* KO mice, and Missler and colleagues, who observed reduced mEPSP frequency in double and triple neurexin KO mice. This difference in result may reflect regional differences in the effects of neurexin1-α. Alternatively, this may be attributable to the genetic background of the mouse line that was studied. The mice used in Etherton et al 2009 were maintained on a mixed SV126/C57BL6J background. The use of the SV126 strain may confound electrophysiological results as this strain possesses additional mutations, including a mutation in DISC1 (Koike et al., 2006). In contrast, the mice that I studied had a "clean" genetic background: they were maintained on a C57BL6J background backcrossed for eight generations to remove background strain effects. In addition, in Missler et al (2003) who worked on double and triple *Nrxn* KO mice, changes in mEPSP frequency may be caused by loss of the other neurexin proteins, which may play a greater role in synapse formation, or may be able to compensate for the absence of neurexin1-α. Indeed, *Nrxn2-α* KO mice display reduced mEPSP frequency (Born et al., 2015b).

The absence of changes in mEPSP frequency suggests that the *Nrxn1α* KO deletion does not alter the number of excitatory synapses, and that neurexin-1α does not play a role in excitatory synapse development. My finding that the evoked EPSP amplitude was not altered

in *Nrxn1α* KO mice is consistent with the suggestion that *Nrxn1α* does not alter the number of excitatory synapses.

The results of this study suggest that in the case of the *Nrxn1α* mutation, there are no deficits in excitatory synaptic pruning, and that accompanying behavioural deficits (e.g. in social behaviour) are not caused by a change in synapse number. Whilst it is possible that there are changes in the number of synapses in other brain regions, the fact that studies of the effects of the *Nrxn1α* deletion in other brain regions, such as the hippocampus (Etherton et al., 2009) and amygdala in rats (Twining et al., 2017), did not find changes in excitatory synapse number supports this claim. Globally, these results suggest that the social dysfunction observed in *Nrxn1α* KO mice is not caused by changes in the number of excitatory synapses or synaptic pruning deficits.

What does this mean with regards to the finding that excitatory synapse number is altered in neurodevelopmental disorders? Neurodevelopmental disorders can have heterogeneous causes, and there are numerous genetic risk factors that increase risk for neurodevelopmental disorders. It is likely that these different risk factors alter circuitry in differing ways. Indeed, some mouse models carrying genetic risk factors do display changes in the number of excitatory synapses (Dani et al., 2005; Dani & Nelson, 2009; Greenough et al., 2001; Harrington et al., 2016; Qiu et al., 2011). It is likely that the exact nature of the developmental disturbances may vary across individuals with neurodevelopmental disorders, and that a change in the number of excitatory synapses may not be a consistent feature across all individuals. This is supported by the small effect sizes observed in neuropathological studies, which do not find changes in number of synapses in all individuals. Whilst this may to some extent account for symptom heterogeneity, it is possible that different circuit abnormalities can produce a similar effect on circuit output.

In this study, I also found no change in mEPSP amplitude and EPSP amplitude, suggesting that post-synaptic AMPA and NMDA receptor function was unaltered in *Nrxn1α* KO mice. Since neurexin-1α is a presynaptic protein, one might not expect a change in post-synaptic function as a result of its loss in neurons. However, neurexin-1 α does bind to post-synaptic proteins, including neuroligins and LRRTMs. Mutations in neuroligins can impair post-synaptic function. It is possible that neurexin1-α could affect post-synaptic function through trans-synaptic signalling via neuroligins and LRRTMs. Indeed, binding of neurexin-3 with post-synaptic proteins has been shown to regulate AMPAR trafficking and post-synaptic function (Aoto et al., 2013). In addition, one study of alpha-neurexin triple KO mice found altered NMDA receptor function in excitatory cells (Kattenstroth, Tantalaki, Sü, Gottmann, & Missler, 2003). However, the results from this study support past research in indicating that *Nrxn1α* does not play a role in post-synaptic function (Etherton et al., 2009; Missler, Zhang, Rohlmann, Kattenstroth, Hammer, Gottmann, & Südhof, 2003; Twining et al., 2017).

In this study, I observed that short-term synaptic facilitation was reduced in *Nrxn1α* KO mice. My findings agree with studies of neurexin-1α function in other brain regions (Missler, Zhang, Rohlmann, Kattenstroth, Hammer, Gottmann, & Südhof, 2003; Twining et al., 2017). In Twining et al (2017), *Nrxn1α* KO rats displayed altered observational learning and absent paired-pulse facilitation in connections linking the medial and lateral amygdala. Reduced facilitation and increased short-term depression have also been observed in other mouse models, including in mice possessing a KO deletion of Nrxn2 (Born et al., 2015b) and numerous other disorders.

My findings suggest that neurexin-1α plays a role in regulating short-term facilitation at excitatory synapses in orbitofrontal cortex. This is likely achieved via its interactions with proteins involved in pre-synaptic vesicle release. Indeed, neurexin-1α interacts with

synaptotagmins, mints and other proteins involved in release. In my study, EPSP amplitude and mEPSP frequency were unaltered in *Nrxn1α* knock-out mice. This indicates that synaptic vesicle release is not abolished by the absence of neurexin-1α. Instead, neurexin-1α may play a specific role in the mechanisms that generate synaptic facilitation. Short-term facilitation requires vesicle release, but likely involves additional mechanisms to those required for low frequency evoked release. Studies have implicated synaptotagmin-7 as a calcium sensor mediating synaptic facilitation (Jackman & Regehr, 2017). It would be of interest to determine whether neurexin-1α binds to synaptotagmin-7.

The loss of short-term facilitation that I describe was not accompanied by a reduction in mEPSP frequency (or amplitude). This may appear surprising given that both depend on presynaptic vesicle release mechanisms. One explanation is that neurexin-1α is involved in the regulation of vesicle release mechanisms that are only elicited by repetitive stimulation. Indeed, short-term facilitation is thought to require additional mechanisms that are not apparent with low frequency activation of synapses.

A different explanation is that different pools of synaptic vesicles generate mEPSPs and action potential dependent synaptic transmission. Traditionally, the vesicles involved in action potential dependent synaptic transmission are thought to be part of a readily releasable pool of synaptic vesicles. Hence, neurexin-1α may play a specific role in regulating vesicle release only from only the readily releasable vesicle pool involved in action potential dependent synaptic transmission.

There has been considerable debate about whether mEPSPs originate from the same pool of vesicles as that used during evoked EPSPs (Kavalali, 2014). Some research suggests that the two originate from pools that can be differentiated. Indeed, spontaneous vesicle release can be blocked without blocking evoked vesicle release, and seem to emanate from a

reluctant/reserve pool (Sara, Virmani, Deák, Liu, & Kavalali, 2005). Spontaneous vesicle release can still occur in mice lacking synaptobrevin, a component of the SNARE complex which is required for calcium-dependent evoked release, although spontaneous release is still decreased (Schoch et al., 2001). Moreover, other SNARE proteins such as VAMP4 and VAMP7 have been found to play a role in spontaneous asynchronous vesicle release. Some research has also found that recovery of spontaneous vesicle release after vesicle depletion relies on refilling of a reserve pool rather than the readily releasable pool, suggesting that the vesicle pools for spontaneous and evoked activity are partially dissociable (Koenig & Ikeda, 1999). However, other research does not support this idea. Indeed, there is a correlation between responsiveness of evoked release and spontaneous release. Moreover, studies have found that the majority (70-90%) of spontaneous events are mediated by synaptobrevin-1, like evoked calcium-dependent release (Xu, Pang, Shin, & Südhof, 2009), and that the same pool of vesicles is used during both spontaneous and activity-dependent release (Wilhelm, Groemer, & Rizzoli, 2010).

I studied the synaptic dynamics of two different excitatory inputs to layer 2/3 pyramidal neurons. Local excitatory inputs within orbitofrontal cortex appeared to exhibit greater short-term facilitation than longer-distance inputs from a different subregion of the prefrontal cortex, prelimbic cortex. The loss of synaptic facilitation in the *Nrxn1α* KO mice may affect the way that information from the different inputs is integrated in orbitofrontal cortex. Reduced facilitation of short-range local input may give greater prominence to longer-range inputs, which may alter the way in which stimuli are processed. Long-range inputs tend to have a longer delay to reach post-synaptic neurons despite their greater myelination. Facilitation of short-range inputs may serve to enhance local inputs and help to integrate local and long-range inputs. Hence, reduced facilitation may lead to reduced temporal integration of inputs.

Synaptic facilitation contributes to the short-term synaptic dynamics and, as such, has been proposed to play a role in information transfer across synapses (Fuhrmann, Segev, Markram, & Tsodyks, 2002). In isolation, synaptic facilitation causes synapses to act as high pass filters of neural firing. This promotes the importance of bursting activity as a mechanism for information transfer (Lisman, 1997). However, in the neocortex, many excitatory synapses exhibit a combination of synaptic facilitation and synaptic depression. When balanced, the synaptic facilitation and synaptic depression counteract each other.

Short-term facilitation has previously been proposed to contribute to aspects of non-social cognition. In particular, short-term facilitation has been proposed to play a role in working memory by preserving certain patterns of activity during a delay (Mongillo, Barak, & Tsodyks, 2008). This is called the synaptic theory of working memory. Since I observed that *Nrxn1α* KO mice display reduced short-term facilitation but intact non-social cognition, my results do not lend support to the synaptic theory of working memory. Instead, my results suggest a role of short-term facilitation in social cognition. Few studies have explored the role of synaptic facilitation in social cognition. However, some lines of evidence support the notion that synaptic facilitation is particularly important for social cognitive functions. First, one recent study indicated that facilitation in the pathway between medial and lateral amygdala may contribute to socially transmitted fear learning (Twining et al., 2017). In addition, reduced short-term facilitation has been observed in a number of rodent models of neurodevelopmental disorders that carry social deficits (see Crabtree and Gogos, 2014, and chapter 6 for a more in-depth review).

In conclusion, my results indicate that *Nrxn1α* KO mice display reduced short-term facilitation in excitatory layer 2/3 pyramidal neurons of the orbitofrontal cortex in the absence of any other changes to excitatory circuitry. This impairment affects both local

connections and long-range connections from the prelimbic cortex, and may have a particularly devastating effect on the function of excitatory circuitry within the orbitofrontal cortex.

4 The role of whisker-mediated sensory input in development of social play

4.1 Introduction

4.1.1 Neurodevelopment in typical and atypical individuals

Neurodevelopmental disorders are a heterogeneous set of conditions that disrupt brain development, resulting in symptoms spanning perceptual, social, cognitive and emotional domains. The high occurrence of neurodevelopmental disorders, which affect 3-4% of children in the UK (Emerson, 2012), suggests that early infant development represents a key period of vulnerability. That early infancy is a vulnerable period is supported by the high incidence of mental illness in children (Green et al., 2005), as well as evidence that early experience plays an important role in the origins of mental illnesses (Beckett et al., 2006; Connor, Bredenkamp, & Rutter, 1999). These studies highlight the need to understand how brain development supports the acquisition of typical and atypical behaviours.

Substantial progress has been made in characterising the trajectory of postnatal brain development and in understanding how it supports perceptual, cognitive and social-emotional development. One line of research has concentrated on the impact of early experiences on cortical specialisation and behavioural development. These studies have revealed "critical" or "sensitive" time-windows during which experiences play a significant role in shaping brain circuitry in a particular brain region. For example, monocular deprivation during early development disrupts the emergence of ocular dominance columns (Hubel & Wiesel, 1964). Likewise, somatosensory deprivation elicited by whisker trimming alters normal barrel cortex connectivity in rat barrel cortex when whiskers are trimmed in the first few weeks after birth (Shepherd et al., 2003). More recently, researchers have begun to explore the impact of early

experiences on cognitive and social development through procedures such as social isolation (Fone & Porkess, 2008). These studies have revealed sensitive periods in development during which social isolation can markedly disrupt social competence in adulthood (Hol et al., 1999; van den Berg et al., 1999).

Investigations into the effects of early experience on development have mostly concentrated on the behavioural domain to which the experience primarily relates. For instance, the impact of early visual deprivation has been assessed in the context of visual development. In contrast, little work has been done to explore whether and how alterations in early experience in one domain affect the development of other cognitive domains. This is important, because the acquisition of new cognitive abilities does not arise in isolation of existing abilities. Multiple studies suggest that the development of at least some complex psychological abilities relies on the normal acquisition of more basic skills. For example, the ability to track and follow eye gaze is thought to be crucial for later social and language development (Senju, Johnson, & Csibra, 2006). As such, altered development in one behavioural domain may influence the course of development of other behavioural domains.

This notion may have particular implications for our understanding of neurodevelopmental disorders. Neurodevelopmental disorders are characterised by the presence of abnormal symptoms across multiple behavioural domains. These symptoms are often considered as distinct problems. For example, the social deficits observed in autism spectrum disorders are studied separately from cognitive or sensory symptoms. However, the interpretation of patterns of "spared" and "impaired" abilities is complex in disorders that occur during development. A genetic or environmental insult that affects the acquisition of one skill or domain may have knock-on "cascade" effects on the later development of other cognitive abilities. Even "spared" abilities may be acquired in a manner that deviates from neurotypical

development (Karmiloff-Smith, 2009; Karmiloff-Smith et al., 2003). Hence, the symptoms observed in neurodevelopmental disorders may be better understood as an end product of a complex interplay between the initial insult, the environment, the effect of the initial insult on continued development, and compensatory changes (Karmiloff-Smith, 2009; Karmiloff-Smith et al., 2003).

According to this "neuroconstructivist" account, a better understanding of neurodevelopmental disorders requires consideration of how cognitive domains interact during development. For example, do early impairments in "lower-level" perceptual or motor domains alter the course of development of other cognitive abilities? It is likely that distinct cognitive abilities differ in the extent to which they rely on that normal acquisition of other abilities.

Social cognition has a particularly protracted developmental trajectory, extending from early childhood into late adolescence and early adulthood (a thorough description of the trajectory of social development is provided in chapter 1, section 1.2.1.). What factors shape social development, and why does social cognition appear so vulnerable to developmental insults? Sensory perception is one factor that may influence social development. Normal social interactions heavily rely on the ability to process sensory cues. During infancy, perceptual development occurs ahead of social cognitive development. Therefore, it is possible that perception may help to shape social development. The aim of this chapter is to investigate this research question.

4.1.2 Social cognition and social development

Social functioning is critical to our survival and health. As a result, we possess dedicated cognitive processes that enable us to navigate social situations, and a rich and flexible

repertoire of social behaviours. In addition, multiple brain regions are involved in processing socially relevant information (Adolphs, 1999, 2001a).

Although certain aspects of social cognition may be present from birth (for example, preference for looking at faces – (Johnson, Dziurawiec, Ellis, & Morton, 1991b), social cognitive abilities are, for the most part, acquired across development. Studies of the trajectory of social development have revealed that distinct social cognitive abilities are acquired at different ages and at different rates (see chapter 1, section 1.2.1). For instance, the ability to follow another person's gaze precedes learning to gesture to attract another person's attention (Tomasello et al., 2007). In addition, the development of at least some social-cognitive abilities appears to build off existing social and non-social skills. For example, the ability to process faces and attend to eyes is required to track gaze, which is an important precursor to joint attention (Farroni, Mansfield, Lai, & Johnson, 2003). Joint attention and pretend play are themselves thought to be important precursors to the acquisition of theory of mind (Charman et al., 2000).

Social cognitive development is particularly protracted compared to other cognitive and perceptual domains; social cognition continues to develop through early infancy into late adolescence and early adulthood. This is mirrored by a protracted period of development of cortical regions that form the "social brain (Huttenlocher, 1979; Huttenlocher et al., 1982; Blakemore, 2008; Petanjek et al., 2011). For example, prominent grey and white matter changes are observed in the prefrontal cortex across adolescence (Barnea-Goraly et al., 2005; Giedd et al., 1999; Gogtay et al., 2004; Sowell, Thompson, Holmes, Jernigan, & Toga, 1999).

As a result of this protracted development, social cognition is particularly susceptible to disruption. Brain injuries and tumours acquired during childhood and adolescence have particularly devastating effects on social cognition. Impaired social cognition is also observed

across numerous developmental disorders. It is a diagnostic feature of autism spectrum disorders (ASDs), but is also a feature of other conditions such as schizophrenia, and William's syndrome (see chapter 1 section 1.1.3). This susceptibility to disruption during development has led to much interest in understanding how social cognitive abilities are acquired during development, and what particular factors shape the course of development.

4.1.3 The role of sensory processing during social interaction

Social interaction relies on sensory input. During social interaction, we extract perceptual information about the external world to make inferences about the social situation and to guide our responses. Perceptual information is used to recognise conspecifics. In addition, we use sensory information to guide more complex features of social cognition. We extract "social meaning" from sensory information to infer other people's intentions and emotional states. Facial expressions, posture and body movement are all potent cues used to recognise the emotions of others (Clarke et al., 2005; Coulson, 2004; Ekman & Oster, 1979). Information from other sensory modalities, such as vocal cues, can also convey emotional content (Scherer et al., 1991). Touch, too, can convey social information and can carry affective significance (Gazzola et al., 2012).

The interplay between sensory processing and social cognition is also reflected in neural circuitry. A number of brain regions that are highly active during in social cognition and constitute part of the "social brain" receive dense input from sensory brain regions. For instance, the orbitofrontal cortex receives input from second-order sensory areas of all modalities (Carmichael and Price, 1995). Likewise, sensory input is projected to the amygdala (Ledoux, Sakaguchi and Reis, 1984; Stefanacci and Amaral, 2002). In addition, some brain regions appear specialised for the perceptual processing of social stimuli. This includes the fusiform face area, which is involved in facial recognition and the superior

temporal sulcus, which plays role in the perception of biological motion (Kanwisher et al., 1997; Thompson et al., 2005).

4.1.4 Sensory processing in neurodevelopmental disorders

Sensory symptoms are highly common in individuals with neurodevelopmental disorders. Although sensory symptoms are not a diagnostic feature of autism spectrum disorders, they are present in a high proportion of individuals with autism spectrum disorder (Ben-Sasson et al., 2009). Estimates for the percentage of individuals with autism spectrum disorder exhibiting sensory symptoms range between 30-100% of individuals (Dawson & Watling, 2000). Sensory symptoms are also observed in other neurodevelopmental conditions. For example, they are seen in children with Fragile X syndrome (Rogers, Hepburn, & Wehner, 2003), and in children with developmental delays (Baranek, David, Poe, Stone, & Watson, 2006).

These sensory symptoms can manifest in multiple forms, and have been most extensively examined in the context of autism spectrum disorder. Both sensory hypo-responsiveness (reduced reactivity to sensory stimuli) and sensory hyper-responsiveness (heightened reactivity to sensory stimuli) have been reported, as well as sensory seeking behaviours. Whilst this partially reflects heterogeneity of symptoms across autistic individuals, disparate responses to sensory stimuli can also be observed within the same individual (Baranek et al., 2006). Given the heterogeneity in sensory symptoms, it is unsurprising that attempts to delve into the nature of the altered sensory processing using objective perceptual tasks have yielded conflicting results; some studies have reported enhanced discrimination and integration in autism spectrum disorders, particularly in the visual domain (Foss-Feig, Tadin, Schauder, & Cascio, 2013). In contrast, other studies have found reduced discriminative abilities (Blake, Turner, Smoski, Pozdol, & Stone, 2003; Manning, Charman, & Pellicano, 2013). Studies that have examined evoked response potentials during sensory processing have likewise observed

considerable variation. For example, one study of auditory evoked potentials in a cross-modal audio-visual study observed enhanced performance in some individuals with autism spectrum disorder, no difference in another subgroup, and hyper-responsiveness to sound and global inhibition of multisensory responses in a third subgroup (Martineau et al., 1992).

Sensory symptoms can be seen across all sensory modalities (Kern et al., 2006; Rogers et al., 2003). However, evidence suggests that tactile and auditory symptoms are the most frequently observed symptoms (Kientz & Dunn, 1997; Wiggins, Robins, Bakeman, & Adamson, 2009). Indeed, tactile symptoms best predict individuals with hyper-responsivity (Kern et al., 2006; Leekam & Ramsden, 2006; Tomchek & Dunn, 2007). Studies investigating the perceptual processes underlying tactile symptoms are also more consistent than for other modalities. These studies indicate that tactile symptoms predominantly take the form of hyper-reactivity to touch, and are accompanied by reduced discrimination thresholds to tactile stimuli (see Robertson and Baron-Cohen, 2017 for a review).

A number of studies suggest that these sensory symptoms may play a critical role in shaping later development and other symptoms in neurodevelopmental disorders. Indeed, sensory symptoms are observed early in neurodevelopmental disorders, emerging prior to overt symptoms and diagnosis. In addition, sensory symptoms predict other features of neurodevelopmental disorders. For example, sensory symptoms are positively related to autism spectrum disorder severity (Adamson et al., 2006; Ben-Sasson et al., 2009; Kern et al., 2007).

Social cognitive development is thought to rely on the acquisition of simple perceptual abilities. Hence, sensory symptoms may play a particularly prominent role in the development of social cognitive impairments. A number of studies support this notion. Sensory sensitivity in children with autism spectrum disorder is associated with greater stress

during social interactions (Corbett, Muscatello, & Blain, 2016). Autistic children with sensory processing dysfunction have lower play scores (Bundy et al., 2007), and sensory abilities predict play performance in autistic children (Kuhaneck & Britner, 2013). The relationship between sensory and social symptoms has been further probed in correlational and regression designs. Whilst some studies have not found an association between social and sensory symptoms, the majority of studies have found a relationship between sensory hypo- and hyper-responsiveness and social impairments has been observed in multiple studies (Hilton et al., 2007; Hilton et al., 2010; Liss et al., 2006; Watson et al., 2011). Atypical touch and olfactory sensitivity have been found to particularly predict social impairment (Hilton et al., 2010). In addition, children with autism display altered neural responses to sensory distractors during social processing tasks indicating greater distraction from sensory stimuli (Green, Hernandez, Bowman, Bookheimer, & Dapretto, 2017).

4.1.5 The rodent juvenile play period

Rats engage in high levels of social behaviours and are a highly social species. Social interactions are vital for their development and health. Social isolation, particularly during development, leads to multiple negative consequences on rodent behaviour and health.

As with human social development, rat social behaviours undergo developmental changes. One of the key developmental periods in rat social development is the emergence of social play (Panksepp, 1981; Thor & Holloway, 1984). Social play consists of reciprocal 'play-fighting' behaviours, characterised by playful attacks and defences of the nape of another animal, which are commonly followed by nibbling and grooming of the nape. The defender can respond by defence or evasion. Frequently, playful attacks ("pounces") and wrestling result in "pins", where the defender animal rotates onto its dorsal surface with the attacker on top. Rough-and-tumble play behaviour is highly reciprocal, and animals will typically switch between being the defender or attacker (Pellis & Pellis, 1991). Play-fighting can be

differentiated from serious fighting in the target of the attack, which tends to be the rump or flank, rather than the nape, in serious fighting (Pellis & Pellis, 1987).

Whilst rodents engage in play behaviours throughout the lifespan, there is a developmental trajectory to play. Play behaviours emerge around P18 and increase in frequency to peak around P30 - P40, declining after puberty at around P60 (Thor & Holloway, 1984). Adult rat interactions are more complex. Whilst adult rats do engage in play-fighting at reduced levels, in adults this is more likely to escalate into dominance behaviours and overt aggression.

Rat juvenile play is highly rewarding to juvenile animals. Juvenile play can be used as an incentive to learn tasks, and can be used to condition place preference (Calcagnetti & Schechter, 1992; Humphreys & Einon, 1981). In addition, rats emit high-frequency ultrasonic vocalisations during appetitive components of play that appear to indicate motivation to play; these vocalisations are emitted more frequently in a chamber that has been previously associated with play, and are emitted in anticipation of play (Knutson et al., 1998a). Rats display approach behaviour upon hearing these vocalisations. This suggests that the vocalisations are associated with reward, and act as communication signals that are associated with play.

Social experience is crucial for rodent social development. Rats that are deprived of social interaction during the peak play period (P21 - P36) display persistent impairments in social behaviour. Rats isolated during development display alterations in social interaction and approach behaviours (Hol et al., 1999). Aggressive behaviours are also altered; isolated rats are more likely to display aggression in non-aggressive contexts, but display impairments in territorial aggression (Potegal & Einon, 1989; van den Berg et al., 1999). There is also evidence that damage to "social brain" regions during the juvenile play period impairs social development, causing a failure to modulate social behaviour according to the context of the

play partner (Pellis et al., 2006). In addition, when placed in a new colony, rats that are deprived of social interaction fail to display normal avoidant behaviours to avoid aggressive attacks from the dominant animal (van den Berg et al., 1999).

This has been shown to be driven by absence of play. Social interaction in the absence of play is not sufficient to rescue behavioural impairments. For example, juvenile rats paired with an anaesthetised partner, or an adult with whom they cannot engage in play, show impaired social behaviour in adulthood (Pellis & McKenna, 1995). Housing with a juvenile separated by a mesh wire is also not sufficient to rescue behavioural impairments (Holloway & Suter, 2004). In contrast, one hour of play experience per day is sufficient to rescue behavioural deficits in isolated juvenile rats. This is not driven by the physical exercise provided by play; providing an isolated rat with an exercise wheel does not restore social behaviour (Holloway & Suter, 2004).

4.1.6 Rodent whiskers and social interaction

Like human social interactions, rodent social interaction relies on sensory processing. The recognition of conspecifics is largely driven by acquisition of an olfactory signature. Odours can also convey information about the sex, identity and dominance status of other conspecifics (Hurst et al., 2001). Auditory calls emitted by other rodents carry information about social situations, and can elicit specific social behaviours depending on the frequency and structure of the call (Knutson et al., 2002; Wöhr, Schwarting, Magnuson, Crawley, & Wilson, 2007).

Rodents also use their whiskers during social interactions. Touch is one of the predominant senses used by rodents to explore their environment. Whiskers form a major source of tactile input for rodents. Rodents acquire detailed tactile information about the external world by performing large-amplitude rhythmic sweeping movements through these whiskers. Tactile

processing via whiskers plays multiple functions for rodents. For example, whiskers are used by rodents to discriminate textures (Carvell & Simons, 1996) and to judge distances (Hutson & Masterton, 1986; Lee, Chen, Chuang, & Wang, 2009).

More recently, the role played by rodent whiskers in social interaction has begun to be studied. Whiskers perform many important social functions. Rodent pups use their whiskers to huddle with their littermates and to locate their mother's nipples for feeding (Arakawa & Erzurumlu, 2015; Sullivan et al., 2003). In adult mice, whisker barbering is an indicator of social dominance and position in cage hierarchy (Long, 1972).

Rodents use their whiskers during direct social interaction. Rats align their faces to whisk each other during social interactions, through large amplitude, irregular whisker movements. These facial interactions using the whiskers are almost as common as anogenital sniffs (Wolfe, Mende, & Brecht, 2011). Whisker movements are pronounced during aggressive encounters. Whisker trimming in adult rats disrupts the ability to engage in normal social interactions, disrupting the ability of the rat to orient itself with respect to social partners, and thereby reducing the frequency of interactions. In addition, whisker trimming in adult rats alters the structure of aggressive attacks, leading to reduced boxing but increased biting. Hence, whiskers are needed for normal social interaction (Wolfe et al., 2011).

The role of social facial touch has also been explored at the neural level. Social facial interactions using whiskers induce membrane potential fluctuations in rodent somatosensory cortex that begin prior to contact (Lenschow & Brecht, 2015). Neurons in the rodent somatosensory "barrel cortex" are strongly activated during whisker social touch, particularly in layer 5b. Neuronal responses are also greater for social touch than non-social touch (for e.g. of an object). In addition, neuron responses in barrel cortex are modulated by the sex of the social partner (Bobrov, Wolfe, Rao, & Brecht, 2014).

In conclusion, it is increasingly clear that whiskers are used during adult social interaction to acquire tactile input. However, these studies have focussed on adult animals. The role of whiskers in juvenile play remains unstudied. One study investigating the contribution of sensory input to socially-induced conditioned place preference indicated that touch acts as a main form of rewarding properties of social interaction (Kummer et al., 2011). However, the precise contribution of whisker touch has not been examined.

4.1.7 Rodent barrel cortex

Tactile sensory input acquired through whiskers project to somatosensory cortex via the thalamus. This information is represented topographically in the primary and secondary somatosensory "barrel" cortices (S1 & S2). Each rodent whisker projects to a group of neurons in an arrangement that parallels the organisation of the whiskers on the rodent's snout (Woolsey and Van Der Loos, 1970).

The barrel cortex develops early. Whilst active 'whisking' behaviour begins at the end of the second week postnatally (Welker, 1964), rats display classical conditioning and response to whisker stimulation in the first week of life (Landers & Sullivan, 1999). This rapid development is mirrored in the cortex. The broad-scale anatomical organisation of barrel cortex is acquired in the first few days after birth (Woolsey & Wann, 1976; Woolsey & Van Der Loos, 1970). Circuitry continues to develop within the different layers of barrel cortex across the next few weeks of life, with layer IV circuitry developing faster than layers 2/3. Layer 4 - layer 2/3 synapses are most plastic around P10 - P14, whilst horizontal layer 2/3 synapses are most plastic between the second and third postnatal week (Maravall, Stern, & Svoboda, 2004; Stern, Maravall, & Svoboda, 2001; Wen & Barth, 2011).

In sum, barrel cortex circuitry displays layer-dependent timeframes of developmental plasticity, but is fairly fixed by P21. Experience-dependent plasticity can continue to be

evoked by whisker trimming in layers 2/3 into adulthood, but this does not lead to large scale disruption of circuitry.

4.1.8 Chapter aims

Social interaction relies on sensory processing. Individuals with neurodevelopmental disorders often possess sensory symptoms, which may play a causal role in social cognitive deficits. However, the extent to which early sensory development shapes social development is unclear. This is difficult to tease apart in individuals with NDDs, where the causes of the condition are complex, little understood and affect many different processes. Addressing the question requires being able to manipulate sensory experience in isolation.

The whisker system is an ideal system to study the effect of altered sensory experience on social development. Whisking is highly active during social contact (Wolfe et al., 2011). In addition, whiskers can be easily trimmed, but regrow rapidly. Hence, it is possible to selectively alter experience within a restricted timeframe.

So for only one study has examined the effect of whisker trimming very early in development (P0 - P10) followed by whisker regrowth on rodent social behaviours. This study has indicated that social interaction is reduced in adult animals after developmental whisker trimming (Soumiya et al., 2016). However, in this study, whisker trimming was conducted at a time that permanently disrupts barrel cortex circuitry. In addition, whisker trimming in this study was conducted before whisking patterns have fully developed; whisking patterns change with age (Arakawa & Erzurumlu, 2015; Grant, Mitchinson, & Prescott, 2012; Welker, 1964). Hence, even when whiskers have regrown, they are unlikely to function normally, meaning that the mice are likely to continue experiencing altered sensation in adulthood. Absence of whiskers in adult mice impairs the ability of rodents to perform their normal social repertoire. This makes it difficult to tease apart social interaction deficits that result

from the effect of neonatal whisker trimming on development from those that arise as a primary consequence of dysfunctional whiskers.

The aim of this study was to investigate the role of somatosensory input in social development. This was achieved by trimming whiskers at an age that does not disrupt barrel circuitry, but that is critical for social development (P21 - P36). By allowing whiskers to then regrow into adulthood, this enabled a dissociation of the immediate effects of disturbed whisker function on the production of social behaviours from effects of the intervention on the development of social abilities. None of the existing studies that have explored the effect of whisker trimming on social behaviour have investigated how social behaviours are altered across different stages of development. Therefore, an aim of this study was to examine social behaviours across development by examining both juvenile interactions, and the effect of the manipulation on social interaction at a later timepoint in adolescence. A third aim of this study was to determine whether the absence of somatosensory input led to enhanced abilities in other perceptual domains that are used during rodent social interaction. To address this aim, I measured olfaction and ultrasonic vocalisations.

4.2 Method

4.2.1 Animals and housing conditions

72 male Sprague-Dawley rats were acquired from Charles River on postnatal day 21. Rats were housed in pairs in cages containing sawdust (Litaspen premium, Datesand Ltd, Manchester), bedding (Sizzlenest, Datesand Ltd, Manchester) and a plastic shelter. Rats were given ad libitum access to food (Rat and Mouse No. 1 and 3 Maintenance Diet for test and breeding mice respectively, Special Diet Services, Essex, UK) and water. Testing rooms and housing rooms were maintained at room temperature (21°C) and 45% humidity. Lighting was maintained on a regular light/dark schedule with lights on between 07:00 and 19:00 hours

(light = 270 lux). Bedding was changed every other week but never on the day of testing. Testing was carried out under licence (PPL: 70/7343; PIL: i28ea4c7f). Behavioural testing was conducted between 08:00 am and 18:00 pm. Although testing rodents during the day may increase noise in behavioural data, previous experiments in our laboratory have indicated that day testing in low light conditions does not significantly alter behaviour in the selected behavioural tasks compared to a non-reverse light cycle.

Bilateral whisker trimming of all whisker rows was conducted on the trim group (n = 36 rats) daily from P21 - P35, which consists of the period during which juvenile play peaks in rats. Control rats (n = 36 rats) underwent daily sham trimming, which consisted of comparable handling whilst stimulating whiskers with the trimming scissors. Housing pairings consisted of either trim-trim (TT, n = 10 pairs, 20 rats), sham-sham (SS, n = 10 pairs, 20 rats) or trim-sham (n = 16 pairs, 16 trim (T[TS]) and 16 sham rats (S[TS])). After trimming, all rats were given a sweet food reward (Sainsbury's Choco Rice Pops, Sainsbury's Supermarket Ltd.).

Tests were conducted in the following order: Juvenile Play, Olfactory habituation/dishabituation (n = 36 rats), Adolescent Social Interactions.

4.2.2 Materials

4.2.2.1 Juvenile Play/Adolescent Social Interactions

Rats were tested in a 40 x 40 x 40 (width x length x height) cm white rectangular arena filled with enough sawdust to cover the base of the arena. Testing was conducted in low light levels (10 lux). A camera positioned over the testing arena and connected to a DVD recorder and TV monitor was used to record rodent behaviours.

Ultrasonic vocalisations were captured using an ultrasound microphone (Avisoft UltraSoundGate condenser microphone capsule CM16, Avisoft Bioacoustics, Berlin,

Germany) sensitive to frequencies between 10 to 180 kHZ. The microphone was connected to a data acquisition device (Avisoft UltraSound Gate 116 Hm) which connected via USB directly into a laptop. Vocalisations were recorded using Avisoft Recorder Software version 4.2. The microphone was positioned in the centre of the testing arena suspended above the arena at a height (65 cm) designed to pick up ultrasonic vocalisations from all angles in the box. A sampling rate of 250 kHz, format 16 bit was used. Avisoft SASLab Pro (Version 5.2) was used for acoustical analysis. A fast fourier transform was performed to generate spectrograms with an FFT-length of 1024 points and a time window of 75% (100% Frame, Hamming window). Frequencies below 20 kHz were removed with a cut-off to remove background noise.

4.2.2.2 Olfactory habituation/dishabituation

Rats were tested in a homecage containing sawdust. Odours were presented on cotton-tipped wooden applicators (approximately 6 inches in length). The wooden applicators were positioned with the cotton tip inserted through the lid of the homecage to a depth of approximately 2.5 cm, and held in place by a clamp secured to a retort stand. The non-social odours consisted of water, banana essence (1:100 dilution in water) and almond essence (1:100 dilution in water). Non-social odours were made up fresh immediately prior to each trial by pipetting 50 µl of the odour onto the cotton-tip of the wooden applicator. For the social odours, cotton-tip applicators were swiped along the bottom of the cage of an unfamiliar rat in a zigzag fashion 12 times. Social odours were stored in a sealable plastic bag until use. Odours were kept outside of the testing room and only brought into the testing room at the start of the trial. Used applicators were placed in a glass waste bottle. Behaviours were recorded using a camera positioned level with the long side of the cage to permit recording of the entire cage.

4.2.3 Procedures

4.2.3.1 Juvenile Play

Rats were tested at P34 and P35. Prior to testing, rats were isolated from their homecage partners for 4.5 hours. For testing, rats were paired with an unfamiliar partner of the same trim status of their homecage partner and their behaviours were observed for 15 minutes. Ultrasonic vocalisations were also recorded for the entire duration of the test.

A subset of rats (n = 36) underwent an additional day of testing on day P36, in which they were paired with a social partner of a different type than that which they were paired with for the previous testing.

4.2.3.2 Adolescent Social Interactions

Rats were tested at P50. Prior to testing, rats were isolated from their homecage partners overnight. For testing, rats were paired with an unfamiliar partner of the same trim status of their homecage partner and their behaviours were observed for 10 minutes. Ultrasonic vocalisations were also recorded for the entire duration of the test.

4.2.3.3 Olfactory habituation/dishabituation

Rats were habituated to the homecage for 10 minutes prior to testing. A clean odourless applicator was placed through the lid of the cage during this acclimatisation period. After this habituation, rats were exposed to sets of odours in the following sequence: water, banana, almond, social odour 1, social odour 2. The two social odours consisted of homecage smells from two different unfamiliar cages. Each odour was presented for three consecutive trials.

Each trial lasted 2 minutes, with a 1 minute inter-trial interval during which the odour stimulus was changed. There were 15 trials in total.

Sniffing of the cotton-tip of the wooden applicator was measured with a stopwatch and scored by an experimenter blind to experimental group. Sniffing was defined as the animal orienting its snout towards the cotton tip at a distance of 2 cm or less away from the cotton bud. Biting on the applicator tip or wooden part was not counted as sniffing.

4.2.4 Statistical Analysis

For juvenile and adolescent animals, play behaviours were measured by scoring the number of pounces and pins performed by each animal during social interaction. A pounce was defined as an attempt to access the partner's nape using paws or snout. A pin was counted when the social partner was rotated, partially or fully, onto its dorsal surface. In addition, the duration of social investigation was measured. The following behaviours were included in the measure of social investigation: social sniffing of the head, body or anogenital region, chasing/following, and social grooming. Results were analysed with a two-way between-subjects ANOVA with independent variables of trim status (trim vs sham) and partner type (same vs different partner).

Olfactory habituation/dishabituation was analysed with a mixed ANOVA with a between-subjects factor of trim status (trim vs sham) and a within-subjects factor of trial. Ultrasonic vocalisations during social interaction were analysed by testing pair, and analysed with a one-way between-subjects ANOVA with an independent variable of pairing (sham-sham, trim-trim, trim-sham).

4.3 Results

4.3.1 Whisker deprivation during the social critical period alters juvenile play behaviour

To investigate the effects of whisker deprivation on social development, play behaviours were first assessed at the end of the whisker deprivation period. This corresponded to the end of the "sensitive period" for the rat play period (P35 - 36).

Whisker trimming did not significantly alter social investigation (Fig. 26A). In contrast, depriving rats of whisker input during the social critical period significantly altered play behaviours. Whisker deprived rats performed a significantly greater number of pounces on their partners than control rats, but only when paired with a whisker deprived partner (trim x partner interaction: $F(1, 68) = 4.581$, $\eta^2 = 0.067$, $p = 0.031$, SS vs TT: $p = 0.012$, S(TS) vs T(TS): $p = 0.757$, Fig. 26B, Table 4). Whisker deprived rats also performed a significantly greater number of pins (sham: 2 ± 1, $n = 36$ rats, trim: 6 ± 1, $n = 36$ rats, $F(1,68) = 4.2$, $\eta^2 = 0.058$, $p = 0.044$, Fig. 26C). Whilst this again appeared to depend on the identity of the social partner, this effect did not reach significance (trim x partner interaction, $F(1, 68) = 3.872$, $\eta^2 = 0.054$, $p = 0.053$, Table 4).

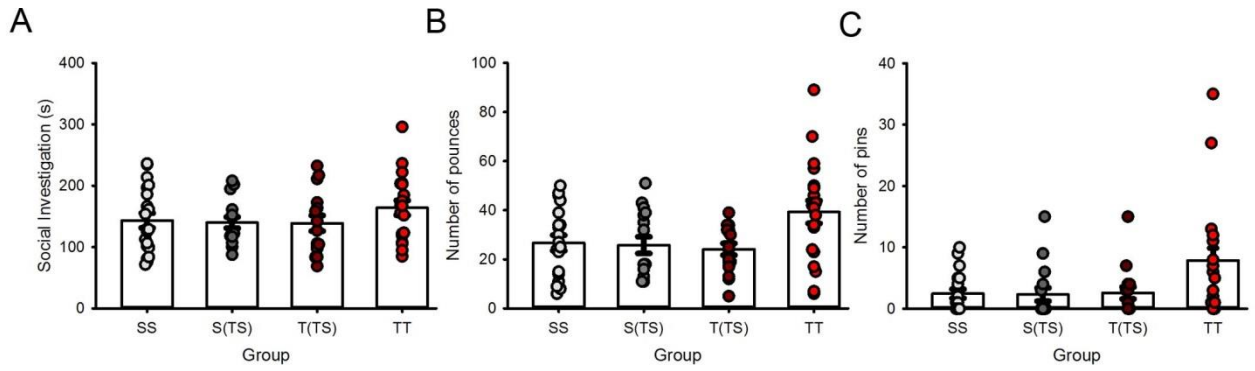

Figure 26: The effect of whisker deprivation during the juvenile play period on play behaviours.

A: Mean ± sem duration of social investigation in sham-sham, trim-trim, trim paired with sham, and sham paired with trim groups, with overlaid scatter. B: Mean ± sem number of pounces in sham-sham, trim-trim, trim paired with sham, and sham paired with trim groups, with overlaid scatter. C: Mean ± sem number of pins in sham-sham, trim-trim, trim paired with sham, and sham paired with trim groups, with overlaid scatter.

Table 4: Juvenile play behaviours.

Mean ± sem number of pounces, pins and mean ± sem duration (s) of social interaction in juvenile rats from sham-sham (SS) pairs, sham rats from trim-sham rat pairs (S[TS], trimmed rats from trim-sham pairs (T[TS]), and trimmed rats from trim-trim (TT) pairs.

Group	Number of pounces	Number of pins	Social interaction (s)
Sham-sham (SS)	27 ± 3	2 ± 1	143 ± 12 s
Sham from trim-sham (S[TS])	26 ± 3	2 ± 1	140 ± 9 s
Trim from trim-sham (T[TS])	24 ± 2	3 ± 1	139 ± 13 s
Trim-trim	39 ± 5	8 ± 2	164 ± 12 s

To further explore the enhanced play observed in the trim-trim group, I investigated the play behaviour of trim-trim rats when paired with a sham animal from the sham-sham group. No

differences were seen in the number of pounces made by the trim rats when paired with a trim or a sham partner ($t(5) = 0.054$, $p = 0.958$, Fig. 27A-C). In addition, although both the duration of social investigation and number of pins made was reduced when trimmed rats were paired with a sham rat, neither change reached statistical significance (social investigation: $t(5) = 1.911$, $p = 0.114$, pinning: $t(5) = 1.939$, $p = 0.110$, Fig. 27A-C, Table 5).

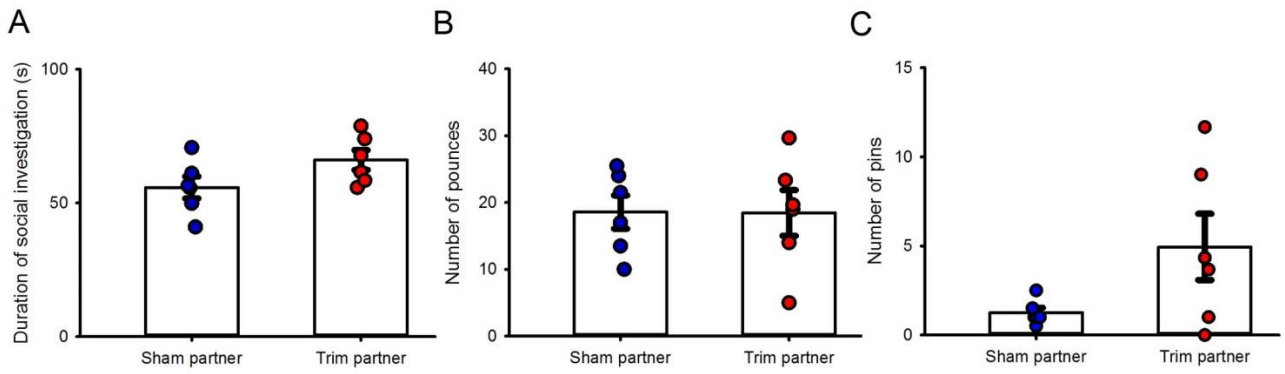

Figure 27: Social investigation and play in trim-trim animals paired with a sham animal.

A: Mean ± sem duration (s) of social investigation in trim-trim animals when paired with a trim versus a sham-sham partner with overlaid, scatter. B: Mean ± sem number of pounces in trim-trim animals when paired with a trim versus a sham-sham partner, with overlaid scatter. C: Mean ± sem number of pins in trim-trim rats when paired with a trim versus a sham-sham partner, with overlaid scatter.

Table 5: Play behaviours for trim-trim animals paired with a sham.

Mean ± sem number of pounces and pins, and duration (s) of social interaction per 5 minutes in trim-trim (TT) rats when paired with a trim versus a sham partner for testing.

Partner Type	Number of pounces	Number of pins	Social interaction (s)
Trim	18 ± 3	5 ± 2	66 ± 4 s
Sham	19 ± 3	1 ± 1	56 ± 4 s

4.3.2 Whisker deprivation during the social critical period leads to long-lasting alterations in play behaviour

I next sought to determine whether deprivation of whisker input during the juvenile play period lead to longer-lasting alterations in play behaviour. To investigate whether the effects on play outlived the whisker deprivation, play behaviours were assessed in adolescent rats (P50), when whiskers had regrown.

Whisker deprivation during the juvenile play period led to lasting effects on social behaviour. This manifested both in social investigation and play behaviours. Rats that had undergone whisker trimming during the juvenile play period displayed a significant increase in the total duration of social investigation compared with sham control rats (sham: 114 ± 7 s, n = 36 rats, trim: 141 ± 8 s, n = 36 rats, $F(1, 68) = 5.838$, $\eta^2 = 0.079$, $p = 0.018$, Fig. 28A, B). In addition, play behaviours were altered in a similar manner to that seen in juvenile rats at the earlier time point. Pouncing and pinning behaviours were increased in rats that had undergone juvenile whisker trimming in a partner-dependent manner (pouncing: trim x partner interaction: $F(1,68) = 5.699$, $\eta^2 = 0.077$, $p = 0.020$, T(TS) vs S(TS): $p = 0.021$; pinning: trim x partner interaction: $F(1,68) = 4.320$, $\eta^2 = 0.060$, $p = 0.041$, Fig. 28C, D, Table 6). Play was selectively increased in the rats that had undergone juvenile whisker trimming and that were paired with a juvenile trimmed partner. Collectively, these results demonstrated that juvenile whisker deprivation led to alterations in social behaviour and play that outlasted the whisker deprivation period by two weeks.

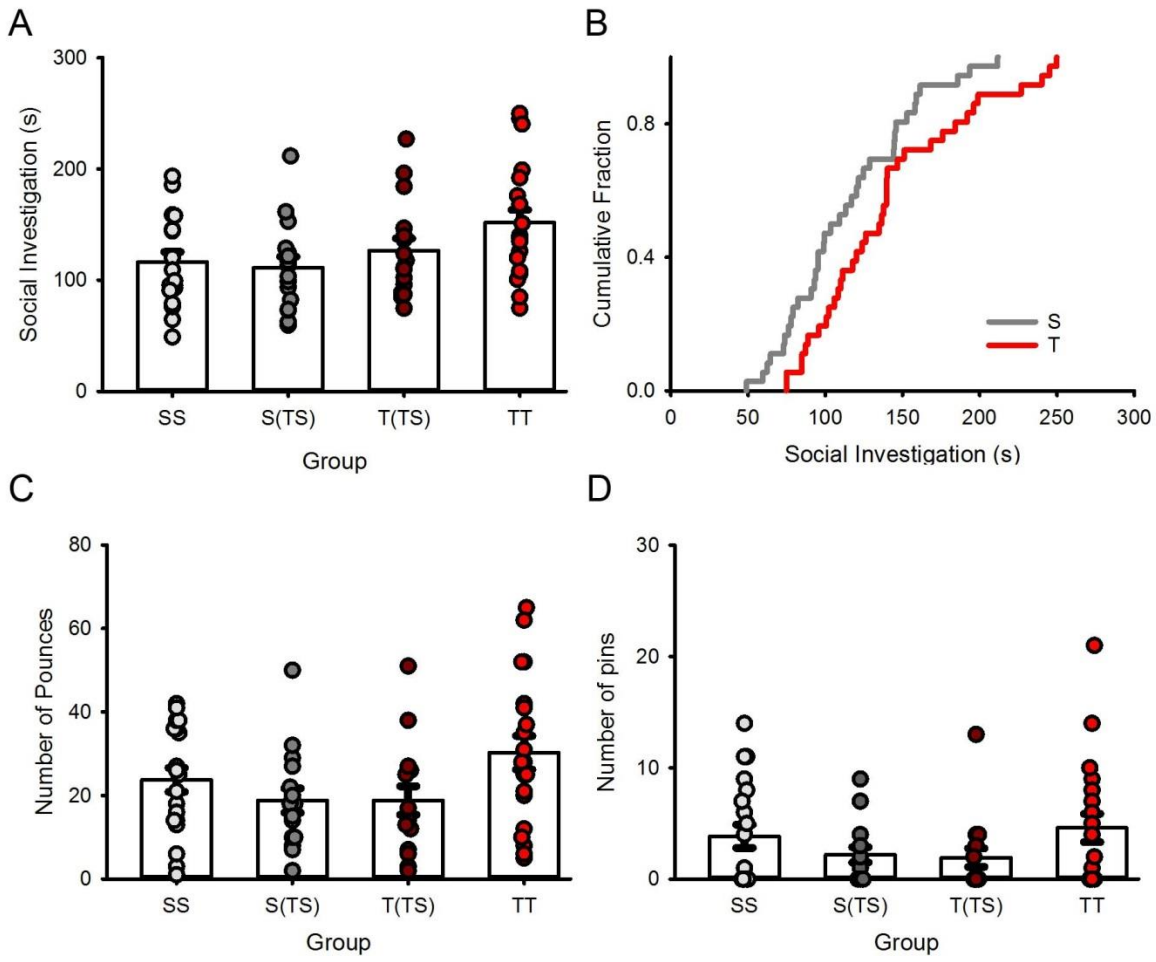

Figure 28: Effects of juvenile whisker deprivation on adolescent social investigation and play. A: Mean ± sem duration of social investigation in sham-sham, trim-trim, trim paired with sham, and sham paired with trim groups, with overlaid scatter. B: Cumulative fraction plot displaying mean duration of social investigation in sham and trim animals. C: Mean ± sem number of pounces in sham-sham, trim-trim, trim paired with sham, and sham paired with trim groups, with overlaid scatter. D: Mean ± sem number of pins in sham-sham, trim-trim, trim paired with sham, and sham paired with trim groups, with overlaid scatter.

Table 6: Adolescent play behaviours.

Mean ± sem number of pounces, pins and mean ± sem duration (s) of social interaction in adolescent rats from sham-sham (SS) pairs, sham rats from trim-sham rat pairs (S[TS], trimmed rats from trim-sham pairs (T[TS]), and trimmed rats from trim-trim (TT) pairs.

Group	Number of pounces	Number of pins	Social interaction (s)
Sham-sham (SS)	24 ± 3	4 ± 1	116 ± 9 s
Sham from trim-sham (S[TS])	19 ± 3	2 ± 1	111 ± 10 s
Trim from trim-sham (T[TS])	19 ± 3	2 ± 1	127 ± 10 s
Trim-trim	30 ± 4	5 ± 1	152 ± 12s

4.3.3 Whisker deprivation during the social critical period does not alter ultrasonic vocalisations (USVs)

Rodents emit high frequency (50-75 kHz) ultrasonic vocalisations during play that act as communicative signals. These calls elicit approach behaviour in other rats and are thought to partially reflect motivation to engage in social interaction. I hypothesised that ultrasonic vocalisations may be altered in whisker-trimmed rats. Indeed, ultrasonic vocalisations may be used to compensate for lack of whiskers as a social signal, or a result of changes in motivation to interact socially.

I sought to determine whether the altered social behaviour observed in rats deprived of whisker input was accompanied by changes ultrasonic vocalisation communication. USVs were measured during the first three minutes of play and compared in different rats pairings (sham-sham, trim-sham, and trim-trim pairs).

First, I investigated whether ultrasonic vocalisations were altered in juvenile rats. There were no differences in the number or duration of USVs emitted across pair groups (USV number:

$F(2,35) = 0.007$, $\eta^2 < 0.001$, $p = 0.994$, USV duration: $F(2,33) = 0.320$, $\eta^2 = 0.019$, $p = 0.729$, Fig. 29A, B, Table 7).

I next investigated whether ultrasonic vocalisations were altered in adolescent rats. As with the juvenile rats, there were no differences in either the number or duration of USVs emitted across pair groups (USV number: $F(2,35) = 1.830$, $\eta^2 = 0.100$, $p = 0.176$, USV duration: $F(2,35) = 0.731$, $\eta^2 = 0.042$, $p = 0.489$, Fig. 29C, D, Table 7).

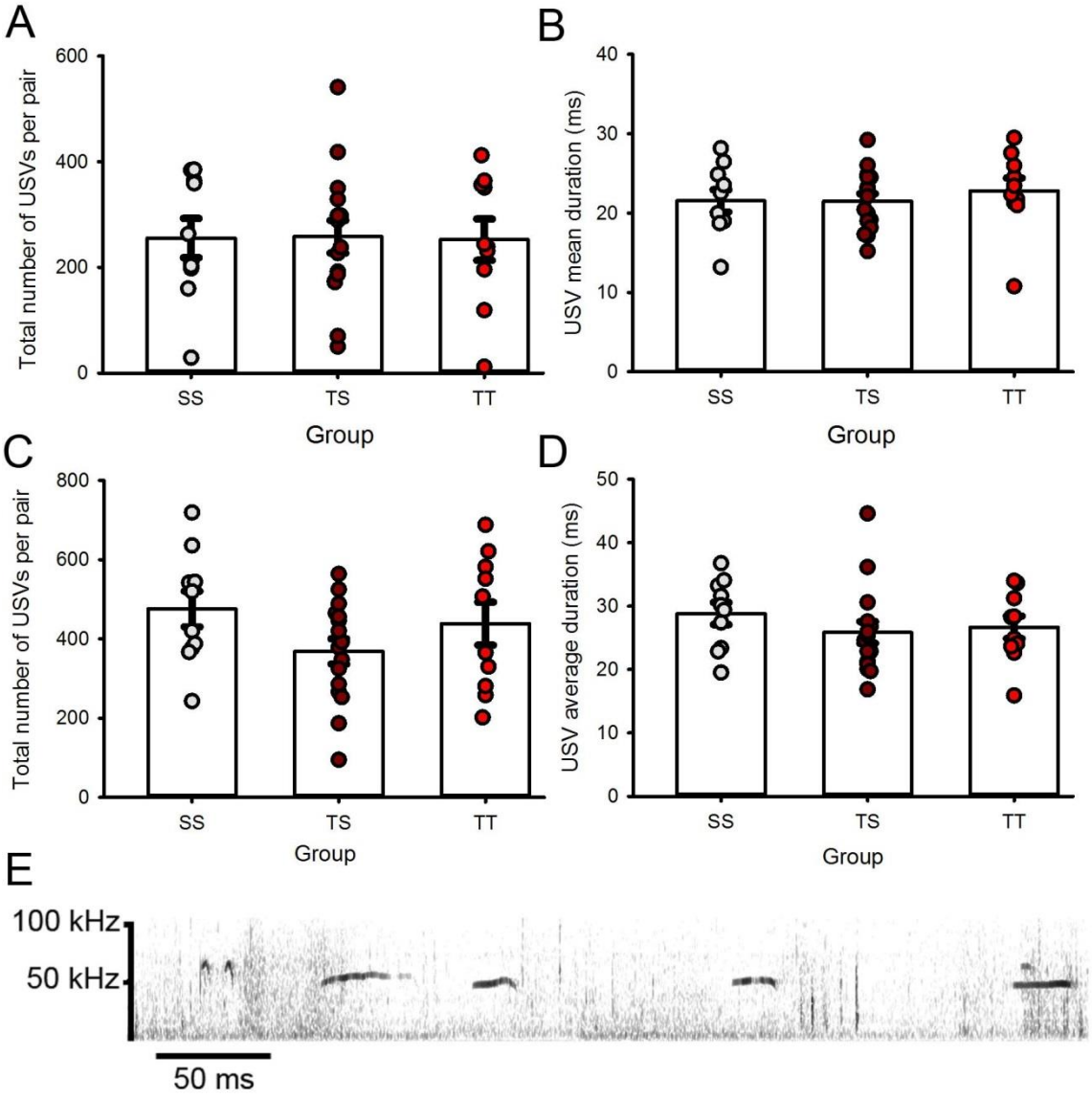

Figure 29: Ultrasonic Vocalisations are not altered by deprivation of whisker input during the play period.

A: Mean ± sem number of USVs per pair in sham-sham, trim-trim and sham-trim juvenile rats with overlaid scatter. B: Mean ± sem duration of USVs in per pair juvenile pairs in sham-sham, trim-trim and sham-trim juvenile rats with overlaid scatter. C: Mean ± sem number of USVs per pair in sham-sham, trim-trim and sham-trim adolescent rats with overlaid scatter. D: Mean ± sem duration of USVs in per pair juvenile pairs in sham-sham, trim-trim and sham-trim adolescent rats with overlaid scatter. E: Example spectrogram trace of ultrasonic vocalisations.

Table 7: Ultrasonic vocalisation data in juvenile and adolescent rats

Mean ± sem number and duration (ms) of ultrasonic vocalisations (USVs) in juvenile and adolescent sham-sham, trim-sham, and trim-trim pairs.

Group	Juvenile USVs		Adolescent USVs	
	Number	Duration (ms)	Number	Duration (ms)
Sham-Sham	256 ± 37	22 ± 1 ms	476 ± 45	29 ± 2 ms
Trim-Sham	258 ± 31	22 ± 1 ms	369 ± 32	26 ± 2 ms
Trim-Trim	252 ± 39	23 ± 2 ms	439 ± 54	27 ± 2 ms

4.3.4 Whisker deprived animals display normal olfactory discrimination in the olfactory habituation-dishabituation task

Rodents possess highly developed olfactory abilities, which are an important component of normal rodent social interaction. Olfactory deficits can confound the interpretation of social behaviour. Hence, it is important to assess olfactory abilities when investigating rodent social behaviour. It is also possible that whisker-trimmed rodents in this study could display heightened olfactory abilities following whisker deprivation. Indeed, in the absence of somatosensory whisker input, rodents may rely to a greater extent on other forms of perceptual input. Following sensory loss in one modality, other perceptual senses may become enhanced through cross-modal plasticity.

In order to determine whether loss of whisker input led to changes in olfactory abilities, rodents were assessed in an olfactory habituation-dishabituation task. There was a significant trial effect ($F(14,462) = 4.282$, $\eta^2 = 0.115$, $p < 0.001$). This indicated that all rodents displayed habituation across multiple presentations of the same odour. There was no significant effect of trim or trim x trial interaction, indicating that whisker trimming during

P21-P36 did not alter olfactory discrimination (trim main effect: ($F(14,462) = 1.165$, $\eta^2 = 0.034$, $p = 0.999$, trim x trial interaction: $F(1,33) = \eta^2 < 0.001$, $p = 0.299$, Fig. 30, for means ± sem see appendix 2, Table 25).

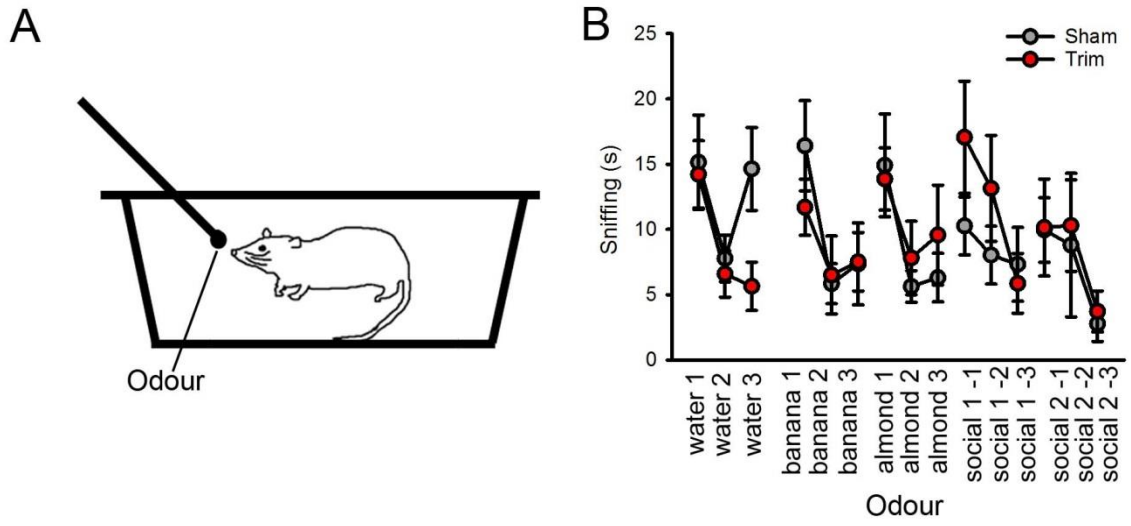

Figure 30: Olfaction is normal in the olfactory habituation-dishabituation task in whisker-trimmed animals.

A: Schematic of the olfactory habituation-dishabituation task. B: Mean ± sem time sniffing of cotton bud in sham and trim rats across trials.

4.4 Discussion

Social cognitive development is protracted, sensitive to experience, and highly vulnerable to disruption. However, the precise factors that contribute to and shape social cognitive development are unknown. Perceptual abilities are one factor that may shape social cognitive development. Perceptual processing is a vital element of social interaction, and sensory symptoms often co-occur with social impairments in individuals who display altered neurodevelopment (see Robertson & Baron-Cohen, 2017 for a review). The aim of this chapter was to investigate the role of sensory input in shaping social development. This was investigated by removing whisker somatosensory input during a period that is critical for

social development (P21 - P36), and allowing whiskers to regrow at the end of this period. I found that removal of whisker input during the juvenile play period altered social play in a partner-dependent manner. These effects were long-lasting and persisted after whisker regrowth. In addition, I investigated whether removal of sensory input in one domain led to greater reliance on other sensory domains to guide social interaction. I found no changes in either olfactory discrimination, or the emittance of ultrasonic vocalisations following whisker trimming.

The first aim of this study was to determine how somatosensory whisker deprivation during the juvenile play period affects social development in juvenile rats. This is the first study to explore the effects of whisker trimming on juvenile play. Indeed, in previous studies, the consequences of developmental whisker trimming for social behaviour have only been examined in adult rats (Soumiya et al., 2016). However, when considering interventions and conditions that are developmental in origin, it is crucial to examine how the whole profile of development is affected (Karmiloff-Smith, 2009). As such, in this study, the effects of developmental whisker trimming on social behaviour were assessed at multiple developmental time points.

The results of this study indicated that juvenile social play behaviours were altered in a partner-dependent manner as a result of whisker trimming during the peak play period (P21-P36). This is the first study to demonstrate a role of whiskers in rodent juvenile play behaviours, and suggests that whiskers may play a role in co-ordinating certain aspects of play.

What role might whiskers play in controlling juvenile play behaviours? The effects found in this study were partner-dependent. Whilst whisker-trimmed rats paired with a similar partner displayed enhanced play, whisker-trimmed rats paired with a sham rat displayed reduced play

behaviours. The fact that trimmed rats paired with a trimmed partner engaged in increased play indicates that the absence of whiskers does not preclude play altogether; rats lacking whisker input are still capable of engaging in play.

One possible explanation for the changes in play observed in this study is that the absence of whiskers reduces the pleasurable properties of play, thereby altering motivation to engage in play. A number of lines of evidence suggest that whiskers may play a role in controlling both the motivation to engage in, and the rewarding properties of play. Play is highly rewarding to juvenile animals and can be used to condition place preferences (Calcagnetti & Schechter, 1992; Vanderschuren, Achterberg, & Trezza, 2016). Social touch may be an important component of the rewarding properties of play. In one study investigating how the different sensory modalities contribute to the pleasurable properties of play, tactile stimulation between partners was necessary in order to condition place preference via play in juvenile animals (Kummer et al., 2011). Studies investigating the effects of tickling on rodents also support this claim; tickling elicits high-frequency "reward" ultrasonic vocalisations that mimic those elicited during juvenile play (Burgdorf & Panksepp, 2001). In addition, tickling, like play, can partially rescue the effects of social isolation on behaviour in juvenile rats (Hori et al., 2014). Human studies also support the notion that social touch is pleasurable and rewarding. Slow-conducting unmyelinated C-tactile (CT) nerves located in hairy skin play an important role in pleasurable human touch (Löken, Wessberg, Morrison, McGlone, & Olausson, 2009; Olausson et al., 2002; Olausson, Wessberg, Morrison, McGlone, & Vallbo, 2010). CT nerves are especially sensitive to gentle touch. Light, pleasurable touch activates brain regions that are involved in reward processing and social cognition, such as the amygdala, STS, medial prefrontal cortex, anterior cingulate and insula (Gordon et al., 2013).

The absence of rewarding input from whiskers could alter motivation to play in two opposing ways. On the one hand, the absence of pleasurable input may reduce the rewarding value of play and decrease the propensity to play. Alternatively, the absence of pleasurable input could lead to increased reward-seeking, and therefore enhanced interest in play in trimmed animals. Whilst this could theoretically account for the behaviour of both trim-trim and trim-sham groups, it does not explain *why* whisker trimming might affect play in opposing directions as a function of housing partner.

Understanding the partner-dependency of the effects requires consideration of what the partner is doing during the interactions. In the absence of whisker input, it is possible that trimmed animals housed together and paired together develop compensatory styles of play and social interaction, relying to a greater extent on other forms of social interaction to provide pleasure. Whilst this study does not support the notion that absence of whisker input leads to compensatory increases in the use of other modalities, such as ultrasonic vocalisation calls, it is possible that the trim-trim group engage in heightened forms of other tactile contact to compensate for absence of whisker-based pleasurable contact. This could lead to enhanced play in trim-trim rats through two mechanisms. First, the use of non-whisker touch to experience the rewarding properties of play could enhance (or keep intact) the motivation to engage in play. Second, increased reliance on non-whisker touch may give the appearance of enhanced play. For example, an increased use of paws may appear similar to pouncing behaviour.

In contrast, trimmed rats housed and paired with a sham rat may not have the opportunity to develop these compensatory strategies. Indeed, the sham rat, possessing intact whiskers, may not develop compensatory behaviours and may continue to attempt to rely on whiskers. The absence of rewarding whisker-to-whisker contact from the trim rat may reduce motivation for

the sham rat to engage in play. As a consequence, motivation to engage in play is also likely to become reduced in the trim rat. The reciprocity of play is important to consider here; if one rat displays reduced motivation to play, the partner can attempt to play but will be unable to engage in successful play bouts. This is likely to lead to the partner also having displaying motivation to play with the test rat.

Other explanations for the reduced play seen in trim-sham rats should also be considered. For example, the absence of whiskers in the trimmed rat might be off-putting to the sham animal (or vice versa). However, if it were simply the absence of whiskers putting rats off engaging in play, the effects would be expected to disappear once whiskers regrew. In contrast, in these experiments, the effects of juvenile whisker trimming on play persisted into adolescence once whiskers had regrown. Importantly, additional experiments indicated that rats from a sham-sham pairing did engage in play with rats from a trim-trim pairing, suggesting that the absence of whisker input *per se* is not off-putting and that the duration of exposure of sham rats to trim rats may be an important factor in determining their behaviour.

An alternative possibility is that the absence of whiskers impairs the ability of animals to engage in reciprocal behaviour. In adult rats, whiskers are important for the ability of rats to orient themselves spatially with respect to other rats during social interaction (Wolfe et al., 2011). The inability to orient themselves might be particularly expected to impair the production of social behaviours that are reciprocal (i.e. as opposed to sniffing, which one animal can engage in without the need for the partner to reciprocate). Since play is highly reciprocal, the absence of whiskers may cause difficulties in engaging in reciprocal play. Whilst this may not preclude play altogether, it may alter the animal's ability to respond to what the partner is doing and hence may alter the structure of their play. Why then, might changes in play be partner-dependent? As proposed above, in trim-trim pairs may be less

affected by a reduced reciprocity of play because both animals may engage in unusual "odd" play. Hence, compensatory strategies to accommodate for altered play may be more easily developed. In contrast, in trim-sham pairs, sham rats may be more put off by unusual play that lacks reciprocity. This may reduce the motivation of both the sham and the trim rat to engage in play.

A second aim of these experiments was to determine whether whisker deprivation during the juvenile play period leads to long-lasting alterations to social behaviours. A key goal was to tease apart behavioural effects that stemmed directly from the absence of whisker input from behavioural changes that reflected a change in social development as a result of absent whisker input.

Perceptual deficits can confound interpretation of social behaviours. Sensory perception guides our social interactions. We use sensory modalities to interpret social gestures and cues during interaction (Clarke et al., 2005; Coulson, 2004; Ekman & Oster, 1979). As a result, impairments in perceptual processing can cause difficulties with certain aspects of social interaction without there being an underlying deficit in social cognition. For example, an individual who loses their sight would struggle to interpret facial expressions, which could lead to more difficulty interpreting people's emotions during interaction. However, this would stem from a visual impairment, rather than from impaired social cognition.

Disentangling changes in social behaviour that arise from impairments in social cognitive processes from those that arise from perceptual impairment can be difficult. This can be particularly problematic when attempting to study the effects of altered perceptual processing on social cognitive development. Are changes in social behaviour a primary consequence of altered perceptual processing itself, or secondary to the effects of changes in perceptual processing on the development of social cognitive mechanisms? This has been an issue for

previous studies that have attempted to investigate this question. A small number of studies have investigated the effects of developmental whisker trimming in mice on later social behaviour. In one study, whisker trimming from birth to P10 led to reduced sociability in the three-chamber task and increased dominance behaviours (Soumiya et al., 2016). In this study, whiskers were allowed to regrow after P10, such that the mice tested in adulthood had fully regrown whiskers. Whilst this may appear to remove the confound of the effects being driven solely by the absence of whisker input, this is not necessarily the case. Whisker trimming from P0 - P10 leads to permanent alterations in barrel cortex circuitry. Whisker trimming during this period alters both the broadscale organisation of "barrels", neuron receptive field properties, and layer IV inputs (Woolsey & Van Der Loos, 1970; Woolsey & Wann, 1976). As a result, although the adult mice may possess fully regrown whiskers, the input acquired through these whiskers is likely to be abnormal. Indeed, adult mice in the study were found to have deficits in tactile perception in a gap-crossing task. Thus, apparent deficits in social behaviour in this study may have been driven by altered perceptual processing. For example, an absence of a preference for the social chamber in the three-chamber task may simply reflect difficulty with navigation and exploration of the chambers.

In the present study, this issue was dealt with by conducting whisker trimming at an age which does not lead to permanent alterations in barrel cortex circuitry and that does not alter whisking patterns, but that is critical for normal social development (P21 - P36). Hence, any effects on social behaviour that persist past the whisker trimming period should reflect an effect on social development, rather than a perceptual processing deficit. In this study, whisker trimming during the "critical" period for social development lead to partner-dependent alterations in play behaviour. The fact that these effects persisted into adolescence, once whiskers had regrown, provides strong evidence that the changes in behaviour were driven by changes to the trajectory of the acquisition of social behaviours. A small possibility

remains that whisker function was still not normal in this study; results would be strengthened by including a task assessing tactile perception. Nonetheless, previous evidence supports the notion that whisker trimming at this age should not alter whisker function. Indeed, whisker discrimination returns rapidly back to normal after whisker trimming post the barrel cortex critical period (Guić-Robles, Valdivieso, & Guajardo, 1989).

A last aim of this study was to investigate the effect of whisker trimming on the use of other sensory signals that may contribute to social interaction. One source of social signal is olfaction. Rodents possess incredibly powerful olfactory abilities. Olfaction and olfactory cues are an important source of social information for rodents. Sniffing is a significant part of their social behavioural repertoire. Olfactory cues carry information about a variety of social contexts, such as partner identity, sex, social status and aggression (Hurst et al., 2001). Therefore, the absence of whisker input may increase reliance on olfactory processing.

This hypothesis was not supported by this study. Whisker trimmed rats did not show any evidence of enhanced olfactory interest in the olfactory habituation-dishabituation task, displaying similar sniffing times to sham rats. In addition, they did not show evidence of enhanced olfactory discrimination abilities in this task. While this suggests that whisker trimmed rodents do not possess enhanced olfaction, it is worth noting that the task used in the current study suffers from limitations. Indeed, in the absence of reward or punishment as an external reinforcer, the olfactory stimuli used in this study may generate limited interest. This could create a floor effect in performance. Using a task in which rats are trained with a positive reinforcer to discriminate between odours would avoid this difficulty.

Ultrasonic vocalisations are another form of sensory cue employed in rodent social interactions. Rodents emit high frequency vocalisations that show sensitivity to social and emotional context (Branchi et al., 2001; Holy & Guo, 2005; Knutson et al., 1998a; Moles et

al., 2007). Lower frequency (22 kHz) vocalisations seem to be evoked in the context of aversive situations, such as during aggressive interactions and fighting, or in anticipation of punishment (Litvin, Blanchard, & Blanchard, 2007). In contrast, higher frequency vocalisations (50 kHz) vocalisations are elicited by rewarding contexts, such as in anticipation of rat play, in response to tickling, and during appetitive aspects of sexual behaviour (Barfield et al., 1979; Knutson, Burgdorf & Panksepp, 1998; Burgdorf & Panksepp, 2001; Willey & Spear, 2012).

I found that whisker trimming did not alter the number of ultrasonic vocalisations produced during social interactions. Hence, whisker trimmed rats did not appear to vocalise more to compensate for the absence of whiskers during social interactions. In this study, ultrasonic vocalisation number could only be analysed at the level of the pair rather than the individual animal, as there was no way to attribute vocalisations to individual animals in the pair. Nonetheless, this is unlikely to much affect results, as we found that both animals in a pair displayed similar play behaviours.

High frequency 50 kHz vocalisations are produced in contexts that are associated with reward. For example, they are produced during play-fighting, during male-female interactions and male ejaculation, in response to stimulation of brain regions that are involved in processing reward (Barfield et al., 1979; Burgdorf and Panksepp, 2001; Burgdorf et al., 2008). They are also produced in anticipation of rewarding contexts, such as in anticipation of play-fighting (Knutson, Burgdorf, & Panksepp, 1998b; Willey & Spear, 2012). This has led to the suggestion that these vocalisations are indicators of positive affect, and may act as an index of motivation (Knutson, Burgdorf, & Panksepp, 1999).

The absence of an increase in ultrasonic vocalisation production in the trim-trim group appears to contradict the idea that these animals display heightened motivation to engage in

play. However, the purpose of 50 kHz ultrasonic vocalisations is still unclear. Although 50 kHz vocalisations are evoked in anticipation of rewards, they do not appear to be uniquely tied to motivation. They are also produced after a reward has been received, and can occur in early stages of aggressive fighting (Burgdorf et al., 2008). They also act as signals that elicit approach behaviours in other animals, leading to the suggestion that they may serve as social signals (Wöhr et al., 2007). Whilst 50 kHz USVs seem to generally reflect positive affect, it is likely that these USVs serve multiple purposes rather than being clearly associated with specific behaviours. Indeed, a recent study suggests that rather than being tied to specific behaviours, high-frequency USVs function as moment-to-moment regulators of social behaviours (Burke, Kisko, Swiftwolfe, Pellis, & Euston, 2017).

The juvenile play period (spanning around P21 - P40) is vital for rodent social development. During this period, rodents engage in intensive "play-fighting" which is thought to enable them to learn how to cope successfully with future adult social interactions and social challenges. The importance of the play period for rodent social development is demonstrated by the profound alterations in social behaviour seen in rodents that are socially isolated during this period (Potegal & Einon, 1989; Hol et al., 1999; van den Berg et al., 1999). The factors regulating and shaping social development during the play period have been unclear. The role of perception in shaping juvenile play has received little attention.

In humans, a number of lines of evidence in humans suggest that social touch plays a role in shaping social development. Touch is one of the first forms of parent-child interaction to emerge (Barnett, 2005). Newborns display responsivity to touch, with tactile stimulation activating a broader network of brain regions that auditory or visual stimulation (Shibata et al., 2012). In addition, 9-month old infants already show sensitivity to pleasant touch, as indicated by changes in heart rate and gaze (Fairhurst, Löken, & Grossmann, 2014). Parental

touch, like facial expressions, modulates infant affect and attention, and can elicit smiling from infants (Stack & Muir, 1992).

The results of this study provide evidence to support the notion that somatosensory input regulates rodent social development. In particular, these results suggest that whiskers are required for the development of normal social motivation and reward mechanisms during the play period. Results from the previous study examining the effects of developmental whisker trimming in mice lend support to our finding; in this study, whisker trimming reduced the motivation to seek social interaction in adulthood (Soumiya et al., 2016). These results also demonstrate that processes governing social motivation and reward develop during the play period. This is consistent with many other studies demonstrating that juvenile play is incredibly rewarding to juvenile rodents (Calcagnetti & Schechter, 1992; Humphreys & Einon, 1981; Trezza et al., 2010). Therefore, successful interactions during this period are likely to be important for later production of affiliative social behaviours.

In addition, multiple studies indicate that experience with play during the play period is vital for the development of adaptive aggressive behaviours, and the ability to modulate social behaviour according to (Pellis et al., 2006; Potegal & Einon, 1989; van den Berg et al., 1999). It would be of interest to determine whether whisker trimming during the play period also interferes with the acquisition of these behaviours. The ability to determine this is limited in the present study; whilst aggressive behaviours can be observed in direct social interaction test, they are likely to be reduced and intermixed with non-aggressive behaviours. A resident-intruder paradigm would be better to address this question. In addition, in the present study, interaction was only assessed in a single context. Assessing behaviour in different contexts (i.e. with aggressive partners or male and female partners) would be of interest to determine whether whiskers are required for contextual modulation of social behaviour.

In conclusion, the results of this study indicated that whisker deprivation during the rodent juvenile play period led to long-lasting, partner-dependent alterations in rodent social interactions. In contrast, whisker trimming did not lead to compensatory changes in ultrasonic vocalisation or olfactory discrimination. The partner-dependent changes in play behaviour observed in this study indicate that whisker trimming does not prevent the ability to engage in play altogether, but may interfere with the acquisition of social reward and motivation mechanisms.

5 The effect of altered sensory experience on the maturation of orbitofrontal excitatory circuitry

5.1 Introduction

5.1.1 Post-natal maturation of sensory circuits

Once established, neuronal circuits undergo a period of post-natal development. This period of maturation is characterised by a rapid increase in synapse formation followed by a slower phase of synapse elimination. In human primary sensory and motor cortices, this rapid increase in synapse formation is most extensive around 3-4 months, with overall synapse number peaking around 1 year of age. At this peak, the total number of synapses is greater than in adult sensory cortex. This is accompaniued by a more prolonged period of synapse elimination until around age 3 which brings total synapse number down to adult levels (Huttenlocher, 1979; Huttenlocher et al., 1982; Huttenlocher, 1990). During development, neuronal connections are further refined by additional mechanisms, including synapse strengthening and weakening, and homeostatic changes (Knudsen, 2004).

Post-natal maturation of neuronal circuitry is sensitive to experience. Studies of post-natal sensory cortex development have demonstrated that there are time "windows" during which experience plays a particularly critical role in shaping circuit development. The absence of normal experience during these "critical" or "sensitive" periods leads to abnormal circuit development and changes in sensory perception. The existence of critical or sensitive periods has been studied extensively in sensory cortices. One of the best characterised examples of critical period plasticity is ocular dominance plasticity. In adults, primary visual cortex is organised in alternating "columns" of neurons that respond preferentially to input from either the right or the left eye. Monocular deprivation in adulthood does not alter the organisation of

ocular dominance columns. In contrast, monocular deprivation during a fixed time window in early development results in the acquisition of abnormal ocular dominance columns. This is characterised by the expansion of columns representing the opened eye at the expense of those representing the closed eye (Hubel & Wiesel, 1964). Since the initial characterisation of critical periods for ocular dominance plasticity, critical or sensitive periods have been described for many other features of sensory processing, such as direction and orientation selectivity in visual cortices, and sound localisation in auditory cortex (Daw & Wyatt, 1976; Knudsen, Knudsen, & Esterly, 1984). Far less work has been done on plasticity in association cortex.

5.1.2 Postnatal maturation of the "social brain"

The term "social brain" is used to refer to brain regions that are particularly active during social cognition. The brain regions typically thought to contribute to the "social brain" consist of the medial prefrontal cortex, anterior cingulate cortex and orbitofrontal cortex, the amygdala, the tempo-parietal junction, the insula and the superior temporal sulcus (Adolphs, 2001; Blakemore, 2008; Brothers, 1996; Frith, 2007).

Researchers have begun to investigate how development of the "social brain" supports social development. Social cognitive development is protracted and extends into late adolescence (Blakemore, 2008). Studies investigating the development of the "social brain" have revealed that, like social cognitive development, the regions comprising the "social brain" mature more slowly than primary sensory and motor cortex. In brain regions associated with more complex cognitive functions such as the frontal lobe, both peak synapse formation and synaptic elimination occur more slowly than in primary sensory areas; in the frontal lobes, peak synapse formation is not reached until 7 years of age. The ensuing decline in synapse number continues into adolescence (Huttenlocher, 1979).

This finding is mirrored by human neuroimaging studes demonstrating that the maturation of "social brain" circuitry continues well into adolescence and early adulthood. A general feature of the neocortex is that grey matter volume follows an inverted "U shape" during development: grey matter first expands beyong normal adult grey matter volume and then shrinks back to stablise at adult levels. In primary sensory and motor regions, grey matter volume is stable after the first few years of life (Gogtay et al., 2004). In contrast, in "social brain" regions, grey matter increases are seen from around 2 years and continue throughout childhood, peaking in pre-adolescence (10-13 years). This is followed by a decline in grey matter volume to reach adult levels.

Different brain regions mature at different rates. Expansion is more rapid in the frontal lobes than the temporal lobe (Matsuzawa et al., 2001). Grey matter volume peaks around 12 years of age in prefrontal and parietal cortex, and 16 years of age in temporal cortex (Giedd et al., 1999). Grey matter volume then declines in the second and third decades of life. Notably, grey matter reductions continue into early adulthood. For example, reductions in grey matter volume continue in post-adolescence in the orbitofrontal cortex and striatum (Sowell et al., 1999).

In addition, long-range white matter tracts also undergo refinement during adolescence. In contrast to the inverted "U shape" of cortical grey matter develoment, white matter density increases linearly during childhood and adolescence up until about 20 years. This is thought to reflect increased myelination of white matter tracts, although other explanations such as increased axonal diameter have also been proposed (Paus, 2010). In particular, increased fractional anisotropy has been reported in the prefrontal cortex, corpus callosum, and striatum (Barnea-Goraly et al., 2005).

Similar patterns of grey and white matter growth are observed in non-human primates. Grey matter volumes increase rapidly around 1-3 months of age, but continue to increase until the late juvenile age. Growth is greatest in the orbitofrontal cortex, where volume peaks at 6.9 months. This is followed by grey matter volume decreases, whilst white matter tracts increase in volume (Uematsu et al., 2017).

Social development is shaped by experience. As with perceptual development, social development is characterised by "sensitive" time windows during which development is particularly shaped by experience. Whilst the precise time window during which experience is most effective varies for different aspects of social development, increasing evidence indicates that adolescence is a senstive period for social development (Blakemore & Mills, 2014). Likewise "social brain" circuits undergo experience-dependent refinement during this period.

The effect of experience on the development of the "social brain" has been extensively investigated in rats. These studies indicate that social isolation during the rat "juvenile play period" (P21 - P40) leads to long-lasting disruption of social behaviour that do not occur when rats are isolated at a later age. Social experience also shapes excitatory circuitry during the juvenile play period. Social experience has been reported to affect synapse number, dendritic branching and dendritic complexity (Bell et al., 2010a; Helmeke et al., 2009). In addition, social experience is required for normal myelination of white matter tracts in the prefrontal cortex (Biro et al., 2017; Liu et al., 2012; Makinodan et al., 2012; Sun et al., 2017).

5.1.3 Maturation of the orbitofrontal and social cognition

Like other brain regions involved in more complex functions, the prefrontal cortex displays a delayed period of synapse formation followed by synapse elimination. The prefrontal cortex undergoes a vast expansion in size in the first few years of life which slowly reduces over

childhood. The orbitofrontal cortex follows a similar pattern of change to the rest of the prefrontal cortex. However, studies have rarely investigated subregions of prefrontal cortex separately to determine whether they mature at different rates. A few studies do indicate that the orbitofrontal cortex may display particularly prominent maturational changes; for example, in one imaging study, volume reductions were observed into the early twenties in the orbitofrontal cortex (Sowell et al., 1999).

Rodent studies have provided further insights into the maturation of orbitofrontal cortex circuitry and its role in social development. These studies have indicated that changes in dendritic length and branching occur in the orbitfrontal cortex during the "juvenile play period". This refinement of circuitry is sensitive to social experience. Social isolation during the developmental play period leads to a reduction in the complexity of dendritic fields in the orbitofrontal cortex (Bock et al., 2008). Orbitofrontal spine development is experience-dependent and influenced by the nature of the experiences acquired during the play period. Animals with more play partners have longer dendrites and more complex basil dendrites (Bell et al., 2010). Octodon degus deprived of paternal care display delayed spine growth in the orbitofrontal cortex (Helmeke et al., 2009). The importance of these changes in circuitry for social development are emphasised by the fact that damage to the orbitofrontal cortex during the "play period" leads to prominent deficits in social behaviour that are similar to those seen in socially isolated animals (Pellis et al., 2006).

The maturation of long-distance connections between the orbitofrontal cortex and other brain regions may also contribute to social development. This has been most explored at the human level through diffusion tract imaging. White matter tracts projecting to the orbitofrontal cortex mature during adolescence, a period during which humans are particularly sensitive to social experience. For example, the uncinate fasciculus linking limbic structures in the

temporal lobe and the orbitofrontal cortex does not mature fully until the third decade of life (Olson, Von Der Heide, Alm, & Vyas, 2015). Changes in myelination in this tract have been related to developing social cognitive abilities (Olson et al., 2015). In addition, a diffusion magnetic resonance imaging study in rodents revealed altered dorsolateral orbitofrontal connectivity in rodents that had undergone social isolation during social development (Liu et al., 2016).

5.1.4 Sensory projections to the orbitofrontal cortex

Projections to the orbitofrontal cortex arise from diverse brain regions. This includes input from the thalamus, hypothalamus, limbic system, temporal lobe, prefrontal cortex (anterior cingulate and medial prefrontal cortex) and from cortical regions involved in sensory processing (see Chapter 1.4.2.1).

The orbitofrontal cortex receives sensory input from all modalities. Olfactory inputs arise from the anterior olfactory nucleus, the piriform cortex, olfactory tubercle and olfactory field of the entorhinal cortex (Morecraft, Geula & Mesulam, 1992; Barbas, 1993; Carmichael and Price, 1995). Gustatory projections are received from the primary gustatory area of the orbitofrontal operculum, adjacent insular field Idg, and ventral area 3. Somatosensory projections arise from ventral primary areas 1-2, secondary somatosensory cortex, insular area Ig and parietal area 7b. Visual inputs stem predominantly from inferior temporal areas TEa and TEp and TEv of the ventral visual streams, as well as areas 7a, 7ip and superior temporal sulcus in the dorsal stream. Finally, auditory inputs originate from multiple primary and secondary auditory areas including primary auditory cortex, and auditory associaiton areas TS1 and TS2 (Romanski et al., 1999).

The extensive projections from sensory regions to the orbitofrontal cortex suggest that the orbitofrontal cortex is involved in processing high-level features of sensory stimuli. This has

been supported by *in vivo* electrophysiological studies in monkeys and neuroimaging studies in humans. *In vivo* electrophysiological recordings demonstrate that populations of neurons in the orbitofrontal cortex respond to gustatory, olfactory, and visual stimuli, and display high selectivity to stimulus identity (Critchley & Rolls, 1996b; Tanabe et al., 1975).

These studies indicate that the orbitofrontal cortex may be particularly involved in representing the reward value of sensory stimuli. Neurons in the orbitofrontal cortex that respond to gustatory stimuli are modulated by hunger and satiety (Critchley & Rolls, 1996a; Rolls et al., 1989). Imaging studies have shown that activity in the orbitofrontal cortex correlated with subjective ratings of pleasantness of tastes (Kringelbach et al., 2003). Moreover, inactivation of the orbitofrontal cortex blocks learned taste aversion in rats (Ramirez-Lugo, Penas-Rincon, Angeles-Duran, & Sotres-Bayon, 2016), and damage to the orbitofrontal cortex impairs taste discrimination, odour-induced taste perception, and pleasurable/aversive responses to taste (Stevenson & Miller, 2013). In trained olfactory discrimination tasks, many orbitofrontal neurons display selectivity to rewarded olfactory cue, or selectivity to odour quality (Critchley & Rolls, 1996b).

Hence, the orbitofrontal cortex may play a role in linking sensory stimuli with reward. This may involve combining information across sensory modalities, as well as combining sensory information with other contextual information that may affect the subjective value of a stimulus. For example, in one study, the orbitofrontal cortex was found to be involved in modulating valuation of taste pleasantness according to other contextual information, such as price (Schmidt, Skvortsova, Kullen, Weber, & Plassmann, 2017).

5.1.5 Touch and the "social brain"

Social touch, that is the touch of one conspecific onto another, acts as a form of communication that conveys social meaning. Gentle, carressing social touch is pleasurable

and signals affection. Therefore, it can be an important part of forming attachments and bonding.

Gentle touch is detected by low-threshold mechanoreceptors in the skin (Zimmerman, Bai, & Ginty, 2014). In the periphery, touch is carried by C type axons. Tactile sensory information projects centrally via the thalamus to multiple cortical maps of the body in somatosensory cortex. The most prominent input is to primary somatosensory cortex, where there is fine-grain, topographic map of the contralateral body. In humans, this map is called the homunculus. In rodents, it is referred to as the ratunuculus. Secondary somatosensory cortex harbours a topographic map of the body, which tends to be coarser than the map in primary somatosensory cortex. Unlike primary somatosensory cortex, secondary somatosensory cortex receives inputs from both sides of the body. In humans, there is a third coarse touch map involving the posterior parietal cortex and insula. In rodents, the homologous map is found in the parietal ventral area (Albieri et al., 2015). Primary and secondary somatosensory cortex are linked by extensive reciprocal connections. Social touch is particularly effective at eliciting neuronal responses in somatosensory cortex; social whisker touch elicits much more neuronal activity than object touch in barrel cortex (Bobrov et al., 2014).

Recently, studies in humans have investigated the effect of somatosensory stimulation on brain regions outside of primary and secondary sensory cortex. These studies have demonstrated that gentle touch that mimics social caressing touch activates a network of brain regions including the bilateral insula, right superior temporal sulcus, bilateral temporoparietal junction, right fusiform gyrus, right amygdala and bilateral ventrolateral prefrontal cortex, including the orbitofrontal cortex. These are the brain regions that constitute the "social brain" (Adolphs, 1999; Blakemore, 2008; Frith, 2007).

The orbitofrontal cortex appears to be strongly recruited during gentle touch. Furthermore, activity in the orbitofrontal cortex appears to be involved in representing the subjective experience and pleasantness of touch. Indeed, multiple BOLD fMRI imaging studies indicate that pleasant touch activates the orbitofrontal cortex (Rolls et al., 2003; Lamm, Silani and Singer, 2015). This is true for both first-hand and second-hand experience of touch (Lamm et al., 2015; McGlone et al., 2012). In contrast, unpleasant touch activates the right fronto-insular cortex (Rolls et al., 2003).

In summary, touch appears to carry social meaning that is processed in the social brain. This is supported by the fact that "social brain" regions are highly responsive to touch. The orbitofrontal cortex is particularly involved in representing the affective properties of touch.

5.1.6 The role of touch in typical and atypical development of the "social brain"

The studies described above indicate that social touch is an important component of social cognition and that "social brain" regions process touch. The response of "social brain" regions to gentle touch develops during the first year of infancy. Gentle touch activates the anterior prefrontal cortex of 10-month old, but not 3 and 6-month old infants (Kida & Shinohara, 2013). As such, it is possible, that social touch contributes to the development of the "social brain". Indeed, the frequency of maternal touch predicts functional connectivity within "social brain" regions including the frontal cortex, insula and superior temporal sulcus in five year olds, suggesting that touch may help to shape development of the "social brain" (Brauer, Xiao, Poulain, Friederici, & Schirmer, 2016).

Altered sensory processing may also contribute to atypical development of social cognition and the "social brain". Individuals with neurodevelopmental disorders frequently display hypo- and hyper-reactivity to sensory stimuli (e.g. see Robertson & Baron-Cohen, 2017 review). These sensory changes are seen early, often preceding diagnosis. As such, it is

possible that sensory dysfunction contributes to atypical development of the "social brain" in individuals with neurodevelopmental disorders. Indeed, BOLD activity within the "social brain" network is hypoactive in response to gentle pleasant touch in children with autism spectrum disorders (Kaiser et al., 2016). Furthermore, autistic traits in "healthy" adults negatively correlate with activity in the orbitofrontal cortex and superior temporal sulcus (Voos, Pelphrey, & Kaiser, 2013). However, the role of sensory experience in shaping maturation of the "social brain" has not been much explored directly.

5.1.7 Aims

Sensory input is not only required for social interactions, but may also shape maturation of the "social brain". This suggests that abnormal sensory processing early in life may disrupt development of the social brain. The effect of altered sensory experience on maturation of cortical circuits has been investigated extensively in primary sensory cortex (Hensch, 2005). In contrast, the effect of sensory experience on neural circuitry in non-sensory association cortex forming the social brain has received scant attention.

The aim of this experiment was to investigate the effect of altered tactile experience on maturation of the "social brain". I focussed on the orbitofrontal cortex, a region that: receives input from multiple somatosensory processing regions; is highly active during social touch; and is involved in processing the rewarding aspects of touch. Sensory experience was altered by whisker trimming rodents during the "juvenile play period", a period during which rodents are particularly sensitive to social experiences, and during which the orbitofrontal cortex undergoes maturation of circuitry.

5.2 Methods

5.2.1 Animal housing and welfare

Procedures were carried out in accordance with the UK Animals (Scientific Procedures) Act 1986 under licence (PIL: 28ea4c7f, PPL: 70/7343 and P431DF110). Animals used in this study consisted of 21 male Sprague Dawley rats obtained from Charlies River Laboratories UK (Margate, UK) that had previously undertaken behavioural testing. Animals were housed in pairs with *ad libitum* access to food and water under a 12 hour dark-light cycle.

5.2.2 Study design

A subset of the rats that underwent whisker trimming and behavioural testing (see chapter 4.2.1) were used for *in vitro* electrophysiological recordings. Rats used in this study underwent either daily bilateral whisker trimming (T) or sham trimming (S) from P21 - P36, followed by behavioural testing. Further details of both the trimming and behavioural testing procedure can be found in chapter 4.2.

Rats were housed in pairs. Either both rats were sham-trimmed animals (SS, n = 10 animals) or both rats underwent bilateral whisker trimming (TT, n = 11 animals).
Electrophysiological studies were conducted on adult rats aged P60 - P80.

5.2.3 Brain dissection and slice preparation

Rats were anaesthetised with 3% Isoflurane (Isoflurane-Vet, Merial Animal Health Ltd, Essex) in 1 L/min O_2. I checked that reflex responses to a painful tail pinch were abolished and then made a midline incision to expose the abdomen, thorax and neck veins. The right and left internal jugular neck veins were cut and I clamped the descending aorta. The tip of the perfusion tube was inserted into the rat's left ventricle and 50 ml of ice-cold dissection artificial cerebrospinal fluid was perfused. The dissection artificial cerebrospinal fluid

contained (in mM): 108 choline chloride, 3 KCl, 26 NaHCO3, 1.25 NaH2PO4, 25 Dextrose, 3 Na pyruvate, 2 CaCl2,1 MgCl2, 285 mOsm, bubbled with 95%O2/CO2.

After transcardiac perfusion, the brain was dissected from the skull and placed in ice-cold dissection artificial cerebrospinal fluid bubbled with 95%O_2/5%CO_2. The brain was glued onto a microtome block and 300 µm coronal slices were cut through frontal cortex using a vibratome (Campden Instruments). Slices were transferred to an incubation bath containing bath-artificial cerebrospinal fluid bubbled with 95%O_2/5%CO_2 and maintained at a temperature of 34 - 36°C for at least one and a half hours before starting to record. The bath-artificial cerebrospinal fluid contained (in mM): (in mM): 124 NaCl, 2.3 KCl, 26 NaHCO3, 1.43 KH2PO4, 10 Dextrose, 1 MgSO4, and 2 CaCl2.

5.2.4 Electrophysiology

Brain slices were transferred to the recording chamber which circulated recording artificial cerebrospinal fluid bubbled with 95%O_2/5%CO_2 and maintained at a temperature of 37 °C containing (in mM): 124 NaCl, 2.3 KCl, 26 NaHCO3, 1.43 KH2PO4, 10 Dextrose, 1 MgSO4, and 2 CaCl2. Slices were held in position using a grating consisting of an arc of flattened platinum wire crossed with strands of dental floss. Slices were visualised at low power using an Olympus BX51W1 light microscope (Olympus UK Ltd) at 2x and 5x magnification.

For electrophysiological recordings, neurons were visualised with a 40x water immersion objective and infrared differential interference contrast (IR-DIC) microscopy. A digital camera (SPOT Insight QE; Diagnostic Instruments Inc., Sterling Heights, MI) and SPOT interface program (Diagnostic Instruments, MI, USA) were used to capture real-time images.

The osmolality of the aCSF was monitored across the day to maintain an osmolality between 285-295 mOsm.

Neurons from ventral and ventrolateral orbitofrontal cortex were patched with recording pipettes pulled from filamented boroscilate glass capillaries (1.5 mm outer diameter, 0.86 mm inner diameter; Harvard Apparatus Ltd., Edenbridge, UK) using a Flaming/Brown micropipette puller (Model P-97; Sutter Insrtument Co., Novato, CA) with a resistance between 4-8 MΩ. Neurons were approached with the pipette in voltage clamp recording mode. During approach onto the neuron, a small positive pressure was applied to the pipette solution to gently blow away surrounding tissue. Recordings were switched to current-clamp mode once a gigaseal had been formed and the membrane inside the pipette tip had been ruptured. Whole-cell recordings were carried out in current-clamp mode using a Axon Multiclamp 700A amplifier (Molecular Devices Corporation, Sunnyvale, CA). Pipette capacitance was compensated (4-7 pF) and bridge balance (10-30 MΩ) was adjusted with the software that controlled the Multiclamp 700A amplifier. Pipette capacitance and access resistance were monitored and adjusted to optimize my recordings. Action potentials were filtered at 10 kHz and synaptic potentials were filtered at 3 kHz using a low-pass 8-pole Bessel filter.

Recordings were discarded if the access resistance exceeded 40 MΩ or if it increased by more than 20%. Neurons with a resting membrane potential more depolarized than -65 mV were discarded. Neurons were selected on the basis of their morphology and a regular spiking pattern of neural firing in response to 500 ms pulses of depolarising currents (400-1000 pA).

5.2.5 Passive membrane properties

Passive membrane properties were measured using the custom written Labview (National Instruments, Austin, TX, USA) program ‘GTF passive membrane properties’. Four traces were averaged per cell for analysis.

Passive membrane properties consist of resting membrane potential (V_m), membrane resistance (R_m), membrane time constant (τ_m) and membrane capacitance (C_m). Resting membrane potential was recorded a few minutes after breaking into the cell. The other passive membrane properties (R_m, τ_m and C_m) of pyramidal neurons was assessed by injecting a 100 ms -100 pA current into the cell. The membrane resistance was calculated using Ohm's law:

$$R_m = \Delta V/I$$

A curve was fitted to the data to calculate the membrane time constant, τ_m. This was calculated as the time taken to reach 1/(1-e) (~63%) of the maximum change in voltage. The membrane capacitance was calculated using the following equation:

$$C_m = \tau_m/R_m$$

5.2.6 Intrinsic excitability

Intrinsic excitability was measured by injecting 500 ms depolarizing current pulses of varying amplitude (+400, +600, +800 and +1000 pA). Smaller currents were injected to identify the minimum amount of current needed to elicit one action potential (rheobase). The number of action potentials was plotted against the amplitude of current injected. I fitted a regression line to the data and calculated the slope and intercept.

5.2.7 Miniature excitatory postsynaptic potentials

Miniature excitatory post-synaptic potentials (mEPSPs) consist of sponaneous release of single vesicles that occur in the absence of an action potential (del Castillo & Katz, 1954; Fatt & Katz, 1952). A more in-depth description of mEPSPs can be found in chapter 3.

mEPSPs were recorded in current clamp mode. To abolish action potential activity, 1µM Tetradotoxin was washed onto the slice. In addition, 100 µM Picrotoxin was washed onto the slice to block $GABA_A$ receptors. mEPSPs were recorded 12-15 minutes after wash-on of these drugs at resting membrane potential. A +1000 pA pulse was applied prior to data collection to ensure that all action potential activity had been abolished. An 8-pole Bessel filter with a -3 dB corner frequency of 3 kHz was used to acquire traces.

mEPSPs were analysed blind to group using the custom written program GTFspontaeousEPSPs v1.2. mEPSPs were measured with two cursors that detected the rise and the peak of the event. Depolarsing potentials were considered to be mEPSPs if they met the following criteria: 1) Time to peak shorter than 5 ms, 2) An asymmetric peak with a decay time longer than the rise time, 3) A peak amplitude that exceeded 2.5 times the root mean square of the noise.

5.2.8 Evoked excitatory postsynaptic potentials

Evoked EPSPs were measured by recording changes in membrane potential in response to axonal stimulation of local axons in layer 2/3. A concentric bipolar stimulating electrode was placed in layer 2/3 ventrolateral orbitofrontal cortex (average distance from neuron: sham: 665 ± 101 µm; trim: 827 ± 100 µm) and a train of 8 pulses was applied using a constant-current generator (Digitimer, Welwyn Garden City). Evoked EPSPs were measured after adding 50µM Picrotoxin (ptx) to the bath to block $GABA_A$ receptors.

Pulses of varying current were applied to determine the current required to elicit an EPSP on all 8 pulses in a train on 50% of trials. This was defined as minimal stimulation. Minimal stimulation was then used to measure evoked EPSP properties and dynamics. A train of 8 pulses lasting 50 µs and at the current required for minimal stimulation was applied at

frequencies of 10, 20 and 40 Hz. Evoked EPSPs were averaged across 10 trials per stimulation frequency.

Normalised EPSP amplitude was calculated as the amplitude of the EPSP response during a train divided by the amplitude of EPSP1, e.g. for the xth EPSP, normalised amplitude = EPSPx/EPSP1. The paired-pulse ratio was calculated as the amplitude of EPSP2 divided by the amplitude of EPSP1. The normalised steady-state amplitude was measured from the normalised EPSP values and was calculated by averaging the amplitude across the last four pulses of the train. Finally, the difference between EPSP2 and the steady-state amplitude was calculated by subtracting the normalised steady-state amplitude from the normalised amplitude of EPSP2. This gave a measure of the depression of responses after EPSP2.

The delay (ms) between stimulation and evoked EPSP activity was measured. The delay to the first EPSP in a train was averaged across stimulation frequencies. To account for differences in electrode placement on the slice and therefore, distance between the electrode and the recorded cell, velocity (m/s) was calculated by dividing the distance between the electrode and the recorded cell by the latency to the first EPSP in a train.

5.2.9 Statistical analysis

Normally distributed data were described by mean ± standard error of the mean (sem) and analysed using parametric tests. A log transform was first applied to non-normally distributed data. If this did not succeed in transforming the data into normally distributed data, the data were described by the median (interquartile range – IQR) and analysed using non-parametric statistics.

Independent t-tests and Mann-Whitney rank sum tests were used to compare two sets of data. For the short-term dynamics data, a mixed ANOVA with independent variables of group

(sham vs trim) and frequency (10, 20, & 40 Hz) was conducted on the normalised data, with a log transform applied where appropriate.

5.3 Results

5.3.1 Passive membrane properties

Passive membrane properties can affect the response of a neuron to current flow across its membrane. Hence, passive membrane properties can affect intrinsic excitability and alter the propensity of the neuron to fire action potentials. Therefore, I first investigated whether a period of whisker deprivation during development affected the passive membrane properties of pyramidal neurons in the orbitofrontal cortex.

Resting membrane potential (Vm) was not significantly different in developmentally whisker-trimmed animals relative to sham animals (sham, Vm = -78 [-76 - -79] mV, n = 15 neurons; trim, Vm = -76 [-74 - -78] mV, n = 19 neurons; Mann-Whitney U = 99.50, p = 0.138) (Fig. 31A). There was also no significant difference in membrane resistance (sham, Rm = 26 [24-32] MΩ, n = 15 neurons; trim Rm = 26 [21-30] MΩ, n = 19 neurons, Mann-Whitney U = 121.00, p = 0.466) (Fig. 31B).

Membrane time constant was similar in sham and developmentally-trimmed whisker animals (sham, τ_m = 9.4 [9.1-10.0] ms, n = 15 neurons; trim τ_m = 9.5 [9.0-10.5] ms, n = 19 neurons, Mann-Whitney U = 121.00, p = 0.466) (Fig. 31C). Finally, membrane capacitance was not affected by developmental whisker trimming (sham, Cm = 358 ± 24 pF, n =15 neurons; trim: 384 ± 24 pF, n = 19 neurons; t-test, t(32) = 0.737, p = 0.467) (Fig. 31D). I concluded that the passive membrane properties of orbitofrontal pyramidal neurons were unaffected by a period of whisker trimming during development.

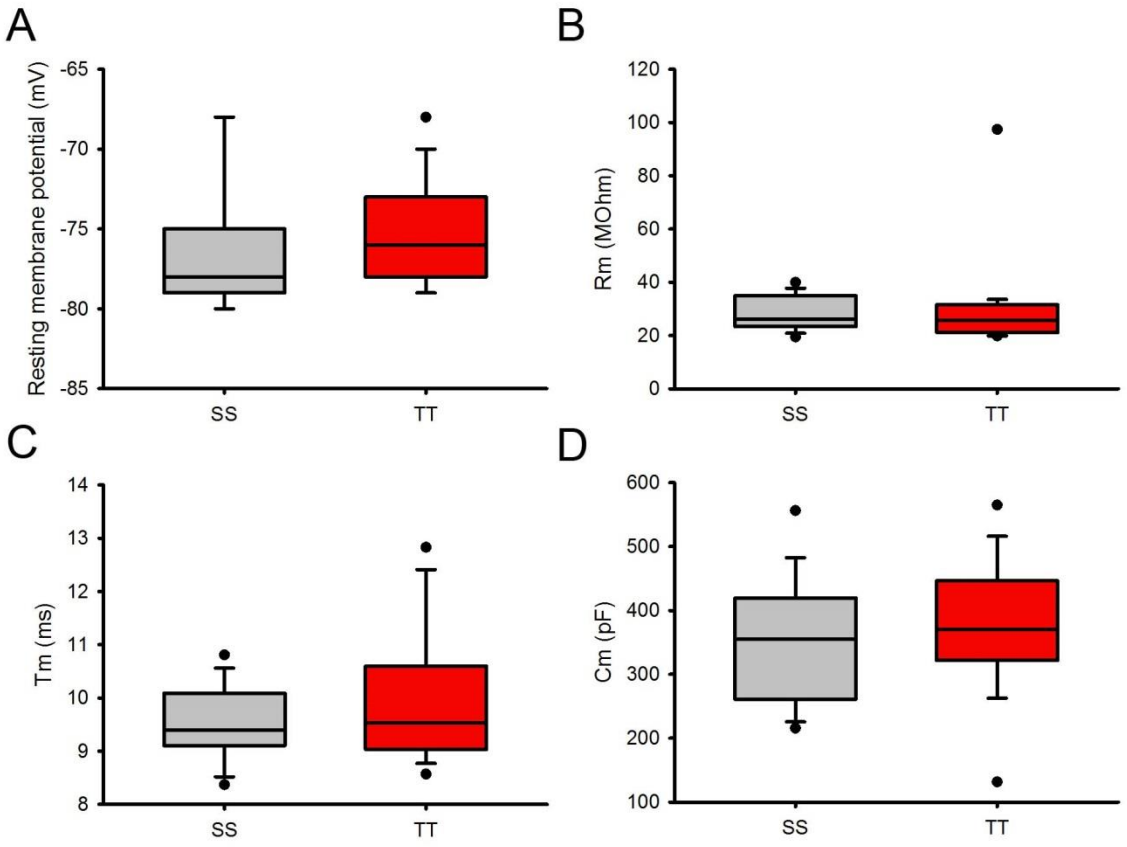

Figure 31: Passive membrane properties of layer 2/3 orbitofrontal pyramidal neurons in sham and developmentally whisker trimmed animals.

A-D: Box and whisker plot of resting membrane potential (A), membrane resistance (B), membrane time constant (C) and membrane capacitance (D) in orbitofrontal pyramidal neurons from sham and developmentally whisker trimmed animals.

5.3.2 Intrinsic excitability

The intrinsic excitability of a neuron is its propensity to fire action potentials and reflects its response to depolarising current flow across the membrane. Intrinsic excitability is sometimes altered during learning paradigms (Sehgal, Ehlers, & Moyer, 2014). In addition, changes in firing patterns and in intrinsic excitability have been described during critical period plasticity (Lambo & Turrigiano, 2013; Maravall et al., 2004). Hence, changes in intrinsic excitability

may occur in the orbitofrontal cortex during the juvenile play period, and may be altered by whisker trimming.

I investigated whether the intrisic excitability of pyramidal orbitofrontal neurons was altered by whisker deprivation during the juvenile play period. I injected depolarising current pulses into layer 2/3 pyramidal neurons in orbitofrontal cortex (Fig. 32A-C). There was no difference in the slope of the input-output function in sham and developmentally whisker-trimmed animals (sham = 0.024 [0.018-0.030] $Hz.pA^{-1}$, n = 15 neurons, trim = 0.028[0.023-0.031] $Hz.pA^{-1}$, n = 19 neurons, Mann-Whitney U = 119.00, p = 0.423) (Fig. 32D. There was also no difference in the constant of the input-output function between sham versus developmentally whisker-trimmed animals (sham: -12.8 ± 2.0 action potentials, n = 15 neurons; trim: -12.7 ± 1.3 action potentials, n = 19 neurons; t-test, t(32) = 0.037, p = 0.971) (Fig. 32E).

Rheobase is defined as the current injection required to elicit one action potential. Rheobase was extrapolated from the input-output function by fitting a line to the data in the range 200-1000 pA and determining the value of x at y = 1. Rheobase was not statistically significantly different in sham and developmentally whisker-trimmed animals (sham: 558 ± 36 pA, n = 15 neurons; trim: 524 ± 37 pA, n = 19 neurons; t-test, t(32) = 0.642, p = 0.525, Fig. 32F). I concluded that the excitability of orbitofrontal pyramidal neurons was unaffected by a period of whisker trimming during development.

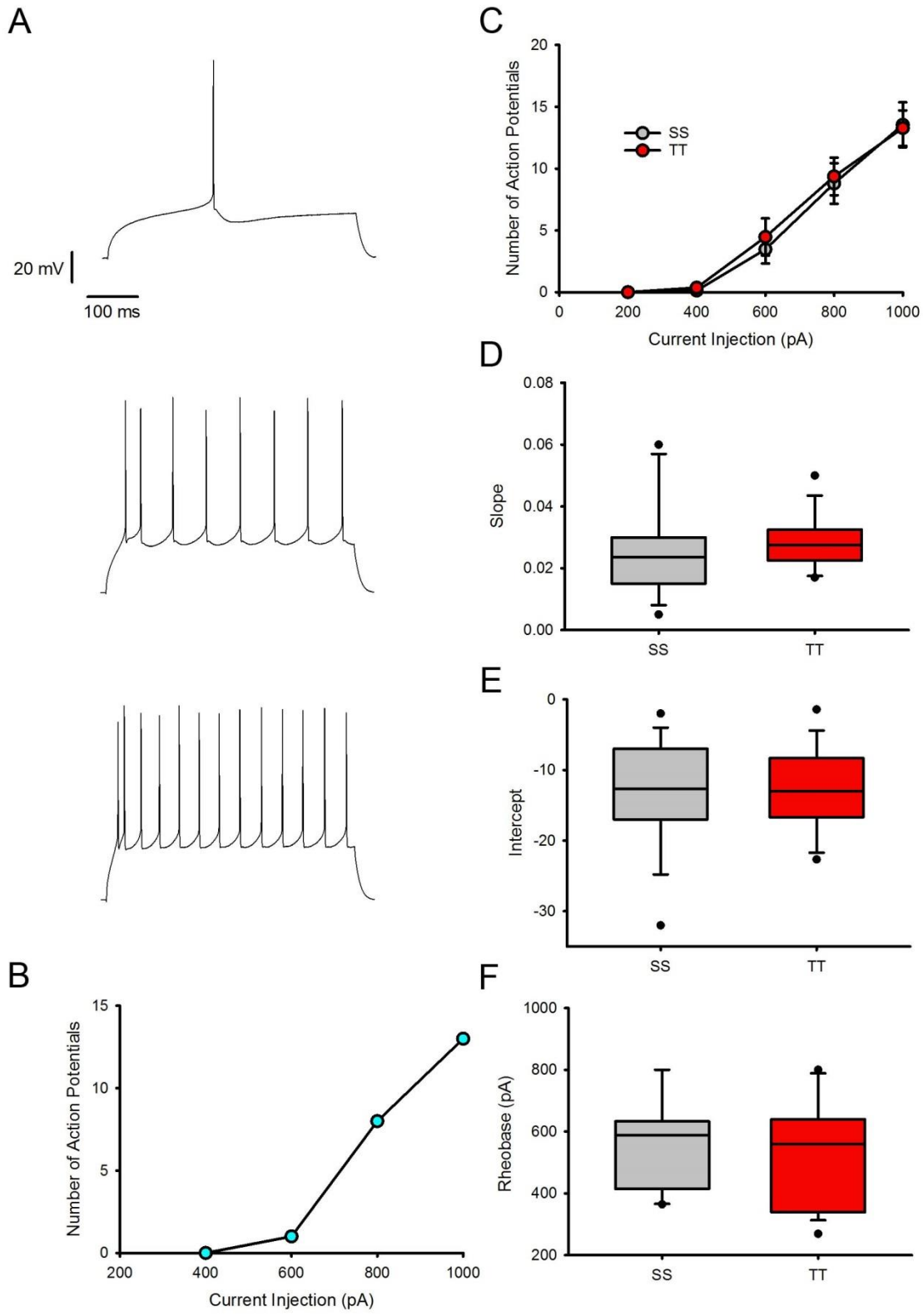
A
20 mV
100 ms
C
Number of Action Potentials
SS
TT
Current Injection (pA)
D
Slope
SS
TT
E
Intercept
SS
TT
B
Number of Action Potentials
Current Injection (pA)
F
Rheobase (pA)
SS
TT

Figure 32: Intrinsic excitability of layer 2/3 pyramidal orbitofrontal neurons in sham and developmentally whisker trimmed animals.

A: Example traces of action potentials in response to 500 ms depolarising current pulses (+600 - +1000 pA) in a layer 2/3 orbitofrontal pyramidal neuron. Scale bars: 100 ms, 20 mV. B: Input-output function for a single orbitofrontal pyramidal neuron. C: Input-output functions for layer 2/3 orbitofrontal pyramidal neurons in sham and developmentally whisker deprived animals. D-F: Box and whisker plots depicting input-output slope (D), intercept (E), and extrapolated rheobase (F) in sham and developmentally whisker deprived animials

5.3.3 Miniature excitatory postsynaptic potentials

Maturation of cortical circuitry involves a period of refinement of neuronal synapses. This is achieved through a rapid increase in synaptic connections, followed by a prolonged period of synapse elimination. In addition, remaining synapses are strengthened.

Changes in sensory experience during the juvenile play period could alter the maturation of synaptic connectivity and function in orbitofrontal cortex pyramidal neurons. I investigated this by measuring the frequency and amplitude of miniature excitatory post-synaptic potentials (mEPSPs) in orbitofrontal pyramidal neurons (Fig. 33A). There was no significant difference in the mean frequency of miniature EPSPs in the orbitofrontal pyramidal neurons of sham and developmentally whisker trimmed rats (sham, 18.7 ± 0.8 Hz, n = 14 neurons; trim, 21.4 ± 1.2 Hz, n = 16 neurons; t-test, $t(28) = 1.863$, $p = 0.073$, Fig. 33A, B)

The amplitude of miniature EPSPs was not significantly different in orbitofrontal pyramidal neurons of sham and developmentally whisker trimmed animals (sham: Vm = 0.15 [0.13-0.16] mV, n = 14 neurons; trim: Vm = 0.15 [0.13-0.16] mV, n = 16 neurons, Mann-Whitney $U = 106.00$, $p = 0.819$, Fig. 33C, D).

I concluded that a period of whisker trimming (P21 – P36) did not affect global excitatory input onto layer 2/3 pyramidal neurons in orbitofrontal cortex.

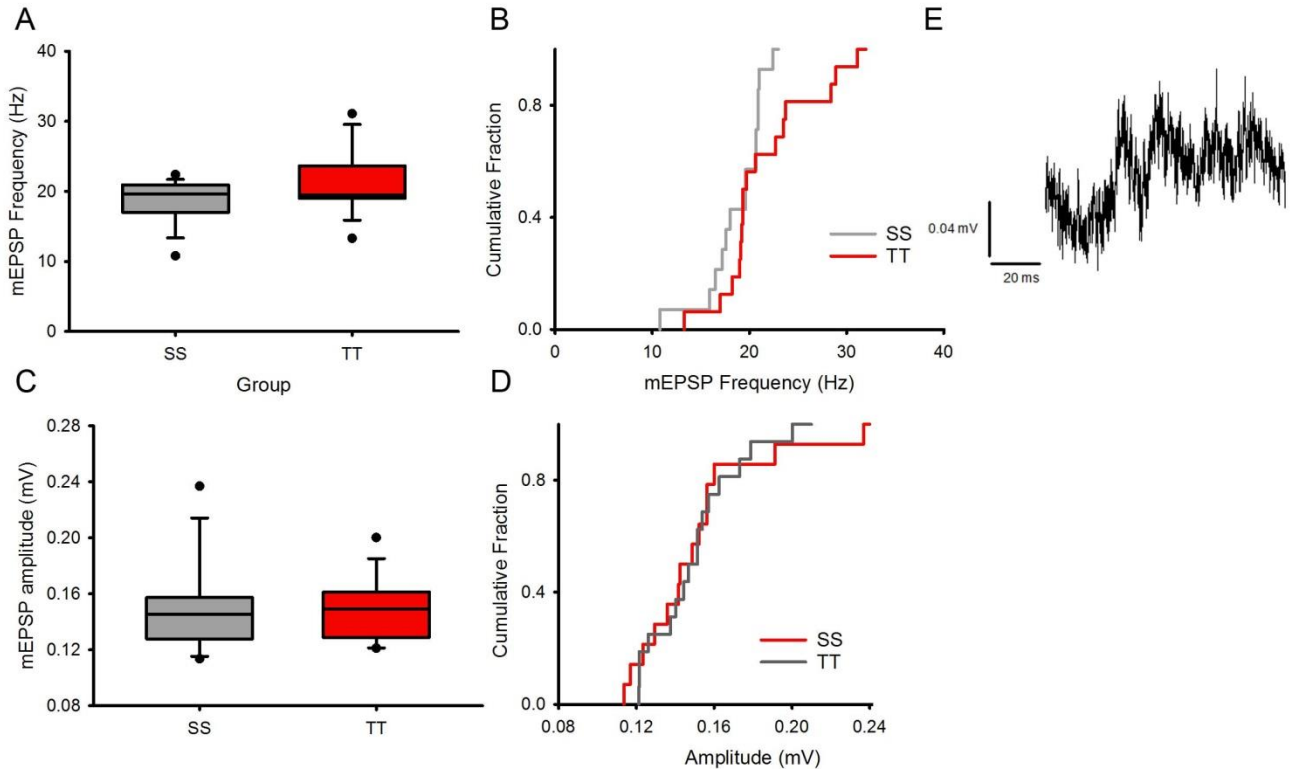

Figure 33: mEPSP properties in sham and developmentally whisker-trimmed orbitofrontal pyramidal neurons.

A, B: Box and whisker plot(A) and cumualtive fraction plot (B) of mEPSP amplitude (mV) in sham and developmentally whisker-trimmed animals. C, D: Box and whisker plot(C) and cumualtive fraction plot (D) of mEPSP frequency (Hz) in sham and developmentally whisker-trimmed animals. E: Example 100 ms trace of recorded spontaneous activity in an orbitofrontal pyramidal cell.

5.3.4 Evoked excitatory post-synaptic potentials

I explored the properties of excitatory connections further in the orbitofrontal cortex of sham and developmentally whisker-trimmed animals by studying evoked excitatory post-synaptic potential (EPSP) activity in response to local layer 2/3 stimulation.

I first investigated the amplitude of evoked EPSPs in orbitofrontal pyramidal neurons in response to minimal stimulation. I used the first response in the train, EPSP1, to measure the response of connections to very low frequency stimulation. I averaged the amplitude of EPSP1 in a pulse train across stimulation frequencies for each neuron. There was no difference in the amplitude of EPSPs in sham and developmentally whisker-trimmed animals (sham: Vm = 0.20 [0.17-0.27] mV, n = 10 neurons, trim: Vm = 0.23 [0.12-0.44] mV, n = 11 neurons, Mann-Whitney U = 52.00, p = 0.860) (Fig. 34A).

Next, I explored the timing of evoked EPSP activity. Myelination in the prefrontal cortex exhibits a critical period, which is dependent on social experience (Makinodan et al 2012; Liu et al 2012), over the same time period that I trimmed rats' whiskers. Therefore, the whisker deprivation protocol could affect the timing of evoked responses by reducing axonal myelination in orbitofrontal cortex.

To investigate the timing of evoked EPSP activity, I averaged the delay between the stimulation artefact and EPSP1 across the three stimulation frequencies. The average latency of evoked EPSP1 in layer 2/3 pyramidal neurons of orbitofrontal cortex was 4.31 ± 0.61 ms in sham-trimmed rats and 4.43 ± 0.37 ms in whisker-trimmed rats. There was no significant difference in conduction velocity between orbitofrontal pyramidal neurons in sham and developmentally whisker-trimmed rodents (sham, 0.16 ± 0.02 $m.s^{-1}$, n = 10 neurons; trim: 0.18 ± 0.01 $m.s^{-1}$, n = 11 neurons; t-test, t(19) = 0.929, p = 0.365, Fig. 34B). I concluded that the whisker trimming protocol did not affect the axonal conduction velocity or latency of synaptic responses within orbitofrontal cortex.

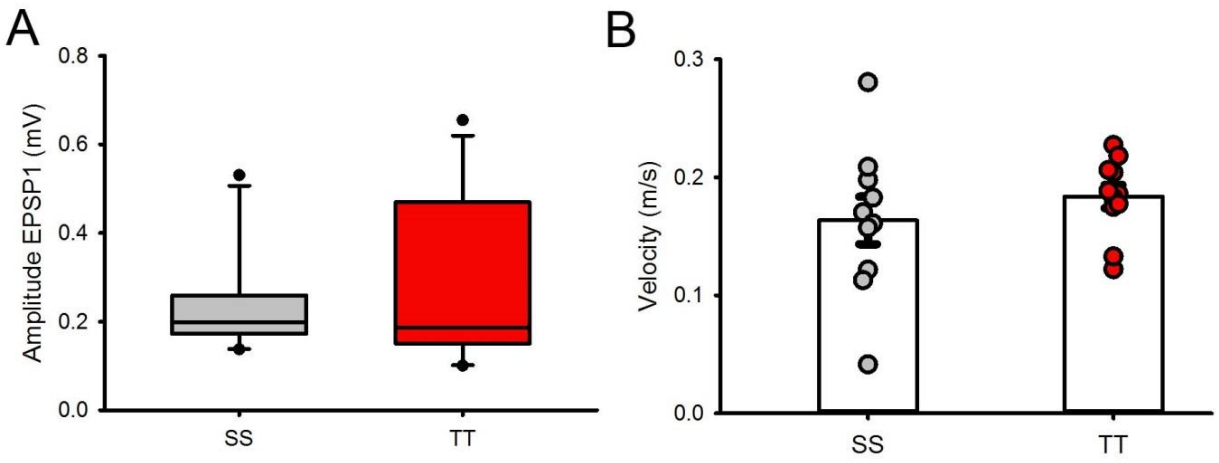

Figure 34: Amplitude and conduction velocity of evoked EPSPs in response to local layer 2/3 stimulation in orbitofrontal pyramidal neurons.

A: Box and whisker plot depicting amplitude (mV) of evoked EPSPs in orbitofrontal pyramidal neurons of sham and developmentally whisker-trimmed rats. B: Mean ± sem conduction velocity (m/s) in orbitofrontal pyramidal neurons of sham and developmentally whisker-trimmed rats with overlaid scatter.

I next investigated the effect of whisker trimming on the short-term dynamics of synaptic responses in layer 2/3 pyramidal neurons of orbitofronal cortex (Fig. 35A). I normalised the amplitudes of all evoked EPSPs to the amplitude of the first pulse (EPSP1) in the train. Evoked EPSPs typically show and initial facilitation followed by depression (Fig. 35B, C). Facilitation peaked early in the train of evoked responses, usually at EPSP2. Therefore, I characterised the initial facilitation by calculating the paired-pulse ratio. As expected, synaptic facilitation was frequency-dependent, with higher frequency stimulation evoking greater paired-pulse facilitation (EPSP2/EPSP1: sham: 10 Hz: 1.24 ± 0.08 mV; 20 Hz: 1.41 ± 0.17 mV; 40 Hz: 2.04 ± 0.49 mV, trim: 10 Hz, 0.95 ± 0.10 mV; 20 Hz: 1.74 ± 0.45 mV; 40 Hz: 1.52 ± 0.41 mV, stimulation frequency main effect: $F(2,38) = 4.221$, $\eta^2 = 0.182$, $p =$

0.022; post-hoc t-tests: 10 Hz vs 40 Hz p = 0.045, log-transformed data). Whisker trimming did not affect the paired pulse ratio displayed by layer 2/3 pyramidal neurons in orbitofrontal cortex (frequency x group interaction: $F(2,38) = 1.317$, $\eta = 0.065$, $p = 0.280$; group main effect: $F(2,19) = 1.426$, $\eta^2 = 0.070$, $p = 0.247$, log transformed data) (Fig. 35D).

After an initial facilitation, synaptic depression became apparent and EPSP amplitude approached a new steady-state (Fig, 35B, C). I measured the normalized steady-state amplitude by averaging the normalized amplitudes of the last four EPSPs in the train. The normalized steady-state amplitude of EPSPs in the train was similar across stimulation frequencies (stimulation frequency: $F(2,38) = 2.433$, $\eta^2 = 0.113$, $p = 0.101$) (Fig. 35E). I found that the steady-state ratio of EPSPs was not significantly different in sham and developmentally trimmed animals ($F(1,19) = 0.970$, $\eta = 0.049$, $p = 0.337$; stim x genotype interaction: $F(2,38) = 1.640$, $\eta^2 = 0.079$, $p = 0.207$) (Fig. 35E).

Finally, I investigated the amount of depression from the second pulse to the end of the pulse by subtracting the normalised steady-state amplitude from the normalised EPSP2 amplitude. There were no significant differences between sham and developmentally whisker-trimmed animals. In addition, the amount of depression from the second pulse to the end of the pulse train was not sigificantly different across stimulation frequencies (stimulation frequency main effect: $F(2,38) = 0.307$, $\eta^2 = 0.016$, $p = 0.737$, stimulation x group interaction: $F(2,38) = 0.857$, $\eta^2 = 0.043$, $p = 0.432$, group main effect: $F(2,19) = 0.011$, eta = 0.001, $p = 0.917$) (Fig. 35F).

I concluded that the whisker trimming protocol did not affect the short-term dynamics of layer 2/3 pyramidal orbitofrontal neurons.

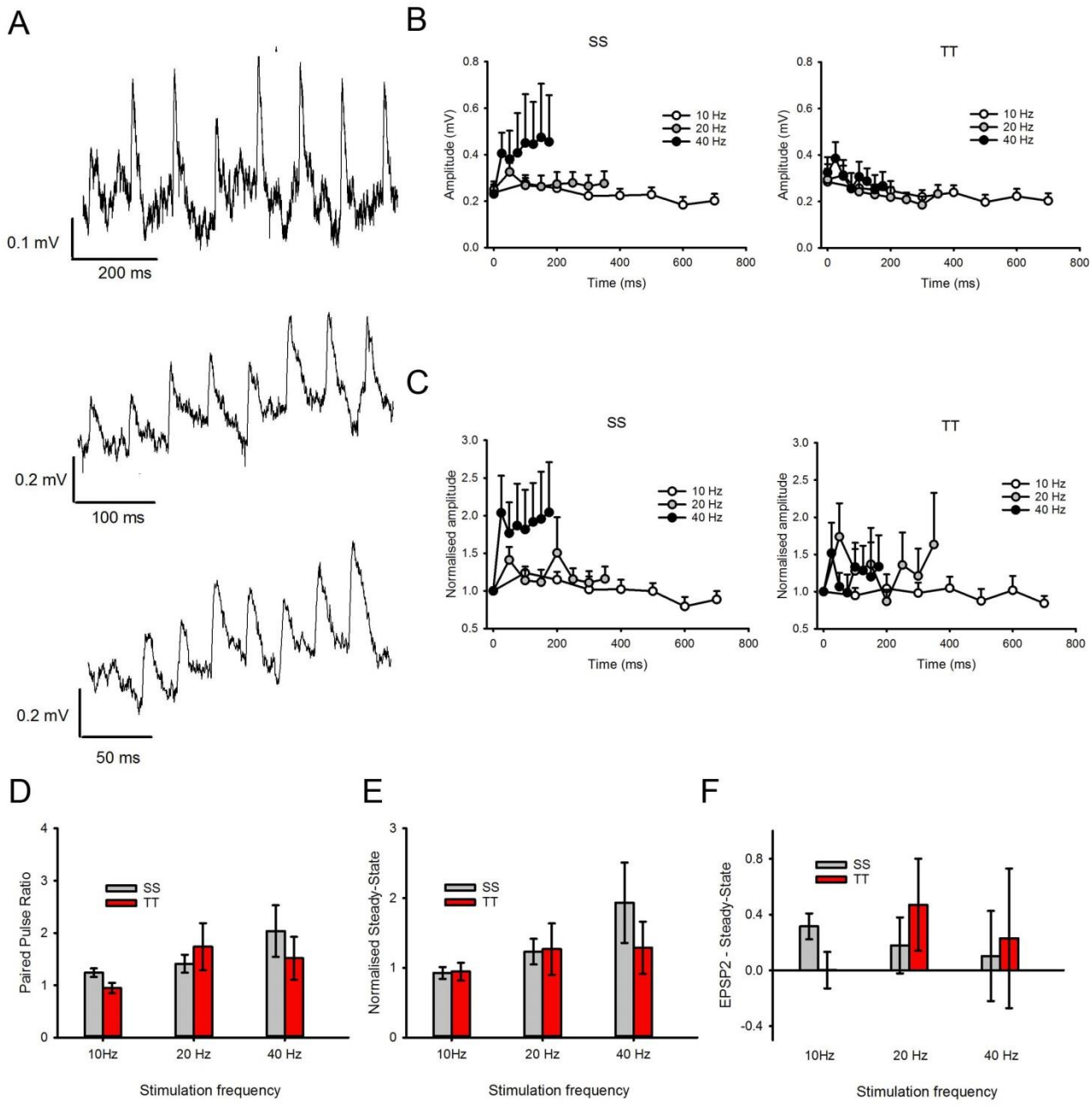

Figure 35: Effect of whisker trimming on the short-term dynamics of evoked EPSPs in orbitofrontal pyramidal cells.

A: Example traces of EPSPs in layer 2/3 pyramidal neurons of orbitofrontal cortex evoked by stimulus trains at 10, 20 and 40 Hz. B: Mean amplitude of evoked EPSPs in pyramidal neurons of sham (left) and developmentally whisker trimmed (right) rodents. C: Mean normalised amplitude (mV) of evoked EPSPs in pyramidal neurons of sham (left) and developmentally whisker trimmed (right) rodents. D:

Mean paired-pulse ratio in pyramidal neurons of sham (left) and developmentally whisker trimmed (right) rodents. E: Mean normalised steady-state in pyramidal neurons of sham (left) and developmentally whisker trimmed (right) rodents. F: Mean difference between EPSP2 amplitude and steady-state amplitude in pyramidal neurons of sham (left) and developmentally whisker trimmed (right) rodents. Error bars B – F, s.e.m..

5.4 Discussion

Sensory input plays a major role in our social interactions. As a result, we possess brain regions dedicated to processing sensory stimuli that carry social meaning, and multiple regions of the "social brain" are activated by social stimuli. A vast body of evidence indicates that sensory experience shapes the post-natal development of primary perceptual processing areas. However, the role of sensory experience in shaping "higher-level" brain regions that are involved in more complex cognitive skills such as social cognition is unclear.

The aim of this chapter was to determine whether changes in tactile experience during the juvenile play period, which is a sensitive period for social development, affected the maturation of excitatory orbitofrontal cortex circuitry. I deprived rats of whisker sensory input during the juvenile play period by daily whisker trimming. My results indicated that juvenile somatosensory deprivation did not lead to changes in either the intrinsic excitability, frequency or amplitude of spontaneous excitatory activity, or the amplitude and short-term dynamics of evoked excitatory activity in orbitofrontal pyramidal cells. Hence, excitatory activity in the orbitofrontal cortex appeared unaffected by a period of complete whisker deprivation during development.

The orbitofrontal cortex is part of a network of brain regions that constitute the "social brain" (Adolphs, 2001; Brothers, 1996). Multiple lines of evidence indicate that post-natal development of the orbitofrontal cortex is important for social development, and that social

experience plays a role in shaping orbitofrontal circuit development. The obitofrontal cortex receives inputs from all sensory systems and is thought to associate sensory stimuli with reward. Hence, one function of the orbitofrontal cortex may be to integrate sensory information for the purpose of social interaction .

I hypothesised that changes in sensory experience during development could affect the maturation of orbitofrontal cortex circuitry. The cellular mechanisms by which experience shapes orbitofrontal development have not been extensively studied. However, the existing studies suggest that changes in excitatory synapses may occur during development (Bell, Pellis, & Kolb, 2010; Helmeke et al., 2009). Hence, I focused on excitatory circuitry.

Multiple studies have reported changes in intrinsic excitability during learning (McKay, Oh, & Disterhoft, 2013). Moreover, changes in intrinsic excitability and in firing patterns have been described during critical period plasticity in the visual cortex and the barrel cortex (Lambo & Turrigiano, 2013; Maravall et al., 2004). Therefore, I investigated whether altered sensory experience affected the intrinsic excitability, passive membrane properties and firing pattern of orbitofrontal pyramidal neurons. I observed no changes in firing pattern, intrinsic excitability, or passive membrane properties as a result of juvenile whisker deprivation. Thus, whisker deprivation during the juvenile play period did not alter the development of pyramidal neuronal intrinsic properties.

Synapse pruning is a second mechanism that may contribute to circuit development during sensitive periods of development. Indeed, post-natal circuit maturation is characterised by a period of a rapid increase in synapse number followed by a slower phase of synapse elimination (Huttenlocher, 1979, 1990; Huttenlocher et al., 1982). This process is delayed in association areas such as the orbitofrontal and prefrontal cortex compared with primary sensory cortex (Huttenlocher, 1979). Synaptic pruning in the orbitofrontal cortex may play a

role in supporting social development. Indeed, the extended phase of synapse elimination partially overlaps with social development; studies indicate that synapse number is still decreasing during adolescence, which has been considered a critical period for social development. Changes in grey matter volume across childhood and adolescence have also been interpreted as indirect evidence for synaptic pruning, with increases and reductions in volume being taken to partially represent synapse formation and elimination, respectively. However, it is worth noting that the period of synapse formation/elimination and grey matter volume changes only partially overlap, making it likely that other processes contribute to reduction in grey matter volume during adolescence. In addition, histological studies examining the orbitofrontal cortex in rodents have failed to detect changes in spine density as a result of altered social experience during the juvenile play period (Bell et al., 2010). However, given that synaptic pruning occurs slowly across a prolonged timescale, it is possible that only small reductions in spine number occur, which could be difficult to detect.

The results of this study indicated that whisker deprivation during the juvenile play period did not affect excitatory synapse density and synaptic pruning in the orbitfrontal cortex. Indeed, I found no difference in either the frequency of miniature excitatory post-synaptic potentials or in the amplitude of evoked excitatory post-synaptic potentials. Both of these measures reflect more than just synapse number, and depend on properties such as the number of vesicles at the pre-synapse, or in the case of evoked excitatory post-synaptic potentials, the strength of synapses. However, the absence of a change in either of these measures provides strong evidence that excitatory synapse number was not affected by tactile deprivation during orbitofrontal development in the juvenile play period.

The experimental data indicate that synapse number is constant on layer 2/3 pyramidal neurons over the critical period for social development. There are no data on synapse

turnover in the orbitofrontal cortex, in large part because it is a difficult area to image longitudinally *in vivo*. It is possible that reorganization of neural circuitry in orbitofrontal cortex relies on a balance between synapse formation and elimination, as has been described in sensory cortices (Barnes & Finnerty, 2010; De Paola et al., 2006; Holtmaat & Svoboda, 2009).

Changes in synaptic strength may contribute to post-natal maturation of social brain circuitry. This may include Hebbian strengthening of synapses, and weakening of synapses that are eventually eliminated (Knudsen, 2004). Few studies have investigated how the strength of connections is altered in the "social brain" during development. This requires electrophysiological investigation, as it is a measure of the function of synapses. However, it is likely that changes in the function of synapses occurs to support social development.

I investigated whether the strength of synapses was altered by sensory experience during the juvenile play period in the orbitofrontal cortex. I found no change in miniature excitatory post-synaptic potential amplitude after whisker deprivation during the juvenile play period. Similarly, the evoked EPSP amplitude in layer 2/3 pyramidal neurons in orbitofrontal cortex was not affected by whisker deprivation. Finally, I found no changes in the short-term dynamics of evoked EPSPs. Taken together, my data suggest that excitatory synaptic transmission in orbitofrontal cortex was unaffected by a period of whisker trimming during development.

At mature synapses, the delay between depolarisation of the presynaptic bouton and the postsynaptic response onset is highly fixed (Katz & Miledi, 1968). However, a recent study indicated that synaptic delay can be altered during experience-dependent reorganisation of circuitry in the barrel cortex (Barnes et al., 2015). Specifically, increases in the latency of synaptic transmission have been reported during periods of increased elimination of neuronal

connections in mature tissue (Barnes et al., 2015). During post-natal brain maturation, circuits undergo a period of prolonged synapse elimination after the initial period of rapid synapse formation (Huttenlocher et al., 1982). Hence, it is possible that the latency of synaptic transmission is more variable during this period of synapse elimination. In addition, post-natal brain maturation is characterised by increases in white matter myelination, which speed axonal conduction. These increases in white matter are particularly notable during adolescence, when brain circuitry is highly sensitive to social experiences (Blakemore, 2008; Makinodan et al., 2012). These changes in myelination are sensitive to social experience during adolescence in the prefrontal and orbitofrontal cortex of rodents; rodents that are socially isolated during adolescence display reduced prefrontal myelination and reduced social interactions (Makinodan et al., 2012; Liu et al., 2016). Finally, the notion that synaptic delay may be more variable during development has been supported by a study reporting changes in the temporal properties of synaptic transmission in the Calyx of Held during development in mice (Fedchyshyn & Wang, 2007).

I investigated whether altered sensory experience during development affected the timing of synaptic transmission in orbitofrontal cortex by measuring the latency between axonal stimulation and postsynaptic evoked EPSP responses. I found no difference in conduction velocity in response to evoked stimulation between neurons in control and developmentally whisker-trimmed animals. This suggested that sensory deprivation during the social sensitive period did not affect the timing of synaptic activity in the orbitofrontal cortex.

In this study, I focussed on the effect of altered sensory experience on the development of excitatory circuitry in the orbitofrontal cortex. Multiple mechanisms have been identified as contributing to critical period plasticity in sensory cortex. The mechanisms underlying development of the "social brain" during sensitive periods of social development have not

been much investigated. Existing studies have pointed to changes in myelination, axonal remodelling and changes in dendritic spine complexity (Bell et al., 2010; Makinodan et al., 2012). However, the precise celluar mechanisms involved have not been much explored, and there has been little electrophysiological investigation of how circuitry in the "social brain" changes during this period. Moreover, there has been no investigation into whether changes in inhibitory circuitry ocur in the orbitofrontal cortex during the social sensitive period. In sensory cortex, changes in GABAergic transmission have been proposed to play an important role in regulating critical period plasticity (Hensch, 2005; Kuhlman et al., 2013). It is possible that this is also true in "social brain" regions too. Likewise, it is possible that changes in sensory experience during the juvenile play period alter the maturation of inhibitory circuitry in the orbitofrontal cortex and other "social brain" regions. This could be explored in future electrophysiological studies.

My findings suggest that changes in sensory experience do not alter the maturation of the social brain. However, there are limitations to the conclusions that can be drawn from my results. The manipulation in this experiment was relatively minor; whilst rodents underwent bilateral complete whisker trimming, they nonetheless received sensory input from other senses, and tactile input from their paws. In addition, the manipulation was only undertaken for a short period (two weeks). It is possible that a more extensive change to sensory experience would have had more of an effect on the development of the social brain. Likewise, it is possible that a manipulation lasting a longer period of time, or starting at an earlier time period, might have had a more dramatic effect. The time period selected in this study was chosen because it corresponds to an important period of rodent social development. However, connections from sensory regions to social brain regions are formed earlier in development, and it is possible that alterations to sensory experience must occur closer in time to this to change social brain circuitry. Finally, it is possible that changes in circuitry did

occur, but were reversed in adulthood once whiskers were returned. Indeed, electrophysiological investigations were undertaken more than a month after the manipulation was terminated. The delay between testing and electrophysiological experiments occurred for unavoidable reasons beyond my control, and were not an intentional aspect of the experimental design. However, this may have given sufficient time for the neural circuitry in orbitofrontal cortex to return to normal.

I concentrated on the orbitofrontal cortex because: it is a region of the social brain that appears to be important for social development in the juvenile play period; it receives extensive sensory input; and it is highly responsive to sensory stimuli. Although changes in sensory experience did not alter orbitofrontal circuitry in a detectable manner, it is possible that other parts of the social brain are altered by abnormal sensory experience. Indeed, in one study, whisker trimming during early development led to increased short-term facilitation, reduced intrinsic excitability and altered AMPA/NMDA ratio in the hippocampus (Milshtein-Parush et al., 2017). Likewise, a separate study found that visual deprivation and whisker deprivation both altered the density of parvalbumin-positive interneurons in the prefrontal cortex and hippocampus (Ueno et al., 2013). It is possible that this reflects regional differences in the effect of sensory deprvation on development. Alternatively, this may reflect differences in the age at which sensory deprivation was conducted; in both previous studies, deprivation was conducted at a younger age.

The aim of this chapter was to investigate whether changes in sensory experience during the social "sensitive period" affected the development of the orbitofrontal cortex, a region that is part of the "social brain". The results of this chapter indicated that tactile deprivation during the social "sensitive period" did not alter the development of excitatory circuitry within the orbitofrontal cortex.

6 Discussion

6.1 Aim of the study

The aims of this thesis were: 1) To investigate the role of dysfunction in the orbitofrontal cortex to impaired social cognition exhibited by mice carrying a genetic risk factor for neurodevelopmental disorders; and 2) To explore the role of sensory input in shaping social development and accompanying circuits involved in social cognition.

My main findings were:

1) *Nrxn1α* KO mice display disturbances of social behaviour. My findings suggest that *Nrxn1α* KO mice could be used to study the neural mechanisms underlying impaired social cognition (Chapter 2).
2) Impaired social cognition in the *Nrxn1α* KO mouse model is accompanied by a reduction in short-term facilitation in layer 2/3 pyramidal neurons of the orbitofrontal cortex that predominantly affects local excitatory connections (Chapter 3).
3) Removal of whisker sensory input during a sensitive window of social development alters social development and causes long-lasting changes in social behaviour (Chapter 4).
4) Removal of whisker sensory input during a sensitive window of social development does not alter the maturation of excitatory circuitry in the orbitofrontal cortex (Chapter 5).

In this thesis, I focused my attention on the role of the orbitofrontal cortex in social cognition. A number of lines of evidence suggest that this structure may be particularly important for social cognition. First, lesion studies in humans, non-human primates, and rodents indicate that damage to the orbitofrontal cortex leads to pronounced and persistent changes in social

behaviour (De Bruin et al., 1983; Grafman et al., 1996; Machado & Bachevalier, 2006; Nonneman et al., 1974). BOLD fMRI studies show that the orbitofrontal cortex is highly active across a range of social cognitive tasks, including tasks assessing violation of social normal, facial emotion recognition, sarcasm detection, and moral judgement (Bas-Hoogendam et al., 2017; Moll et al., 2002; Uchiyama et al., 2006). Moreover, alteration in both structural and functional imaging have been observed in the orbitofrontal cortex across a number of neurodevelopmental conditions displaying social cognitive impairments, including autism spectrum disorders, schizophrenia, William's syndrome, and Turner's syndrome (Girgis et al., 2007; Meda et al., 2012; Mimura et al., 2010). Finally, social cognitive development in both humans and rodents is accompanied by grey and white matter changes in the orbitofrontal cortex (Olson et al., 2015; Liu et al., 2016).

Nonetheless, few studies have examined the contribution of the orbitofrontal cortex to social cognition at the cellular level. *In vivo* single-cell electrophysiological studies in the orbitofrontal cortex have concentrated on non-social decision-making tasks (Schoenbaum & Eichenbaum, 1995a; Tremblay & Schultz, 2000). In addition, electrophysiological studies investigating circuit abnormalities in mouse models of neurodevelopmental disorders have rarely concentrated on the orbitofrontal cortex; only one previous study has recorded in orbitofrontal cortex (Jiang-Xie et al., 2014).

I concentrated my investigation on excitatory circuitry in the orbitofrontal cortex. Disturbance of excitatory circuit development and function may be evident in the pathology of neurodevelopmental disorders. Indeed, post-mortem neuropathology studies reveal dendritic spine abnormalities in the brains of individuals with autism spectrum disorders, schizophrenia, and Fragile X syndrome (Garey et al., 1998; Glantz & Lewis, 2000; Harrison & Eastwood, 1998; Hutsler & Zhang, 2010; Irwin et al., 2001; Kolluri et al., 2005). Altered

glutamate levels have also been reported in these disorders (Harrison & Eastwood, 1998; Purcell et al., 2001; Shinohe et al., 2006). In addition, many of the genetic abnormalities that increase risk neurodevelopmental disorders affect excitatory circuitry (Alarcón et al., 2008; Arking et al., 2008; Durand et al., 2007; Fromer et al., 2014; Gauthier et al., 2009, 2010, 2011; Jamain et al., 2003; Kim et al., 2008; Laumonnier et al., 2004; Strauss et al., 2006; Vaags et al., 2012). As such, excitatory dysfunction in the orbitofrontal cortex may contribute to impaired social cognition in neurodevelopmental disorders. Indeed, social development during the rodent juvenile play period is accompanied by dendritic spine changes in the orbitofrontal cortex (Bell et al., 2010; Bock et al., 2008). Moreover, rodents that are exposed to altered social experiences during this period display altered dendritic spine properties in the orbitofrontal cortex compared to typically developing animals (Bell et al., 2010; Bock et al., 2008).

I focussed on layers 2/3 of the orbitofrontal cortex. Layers 2/3 are the main source of cortico-cortico inputs. Orbitofrontal cortex layers 2/3 receive long-range inputs from many cortical regions, as well as from the amygdala, which must be integrated with local orbitofrontal cortical neuronal connections (Morecraft, Geula and Mesulam, 1992; Carmichael and Price, 1995b; Rempel-Clower and Barbas, 1998). Alterations in layer 2/3 may contribute to social impairment in neurodevelopmental disorders. Indeed, changes in dendritic spine number or shape reported in autism spectrum disorders and schizophrenia were found in cortical layers 2/3 (Glantz & Lewis, 2000; Hutsler & Zhang, 2010). In addition, recent theories of autism spectrum disorder and schizophrenia have proposed that these conditions are characterised by an altered balance of long and short-range inputs (Courchesne & Pierce, 2005).

6.2 Factors contributing to social cognition in typical and atypical development

Social cognition has a protracted trajectory of development that extends from early infancy through to late adolescence and early adulthood (Choudhury et al., 2006) (see Chapter 1.2.1). As a result, social cognition is vulnerable to developmental insults. Brain damage acquired during childhood leads to particularly devastating impairments in social cognition (Ryan et al., 2014). Impaired social cognition is also a common feature across multiple neurodevelopmental disorders, including autism spectrum disorders, schizophrenia, William's syndrome, Turner's syndrome, and various other syndromic conditions (e.g. Prader-Willi syndrome, Angelman syndrome, Fragile X syndrome). Hence, it is vital to understand the factors that influence social cognitive development in health and in disease.

6.2.1 Genetics and social cognition

Multiple lines of evidence indicate that genetics plays an important contribution to social cognition. Twin studies comparing correlations in monozygotic and dizygotic twins indicate that multiple aspects of social cognition possess substantial heritability, and that the heritability of social abilities increases during development. For example, the heritability of prosocial behaviour increases from around 26-37% at age 2 to 51-72% at age 7 (Ariel Knafo & Plomin, 2006). An increase in heritability during development is also observed for empathy, with heritability estimates reaching 37-47% at 2-3 years (Knafo et al., 2008). Substantial additive genetic influences have also been observed in other facets of social cognitive behaviour. For instance, 32% of the variance in antisocial behaviour is accounted for by additive genetic influences (Rhee & Waldman, 2002). Co-operative social decision-making is also highly heritable; 40% of the variation in performance in the ultimatum game (Wallace, Cesarini, Lichtenstein, & Johannesson, 2007), and 25% of variation in performance in dictator game is accounted for by additive genetic influences (Cesarini et al., 2009).

The role of genetics in social cognition has been further uncovered through the exploration of specific genetic variants that influence social behaviour in humans (e.g. through GWA

studies). Such studies have uncovered a number of genetic variants that have been consistently associated with social performance. For example, the length of the repeat region in AVPR1α (the AVP receptor gene) is related to multiple aspects of social behaviour, including altruism in the Dictator game (Knafo et al., 2008), pair bonding (Walum et al., 2008), the strength of social/family relationships (Bachner-Melman et al., 2005), and BOLD activity in the amygdala during emotion recognition tasks (Meyer-Lindenberg, 2008). Other genes that have been associated with social behaviour in humans include the gene encoding the oxytocin receptor (OXTR), the gene encoding monoamine oxidase A (MAOA) which is responsible for neurotransmitter degradation, and the gene encoding for the serotonin transporter (SLC6AF). Genetic polymorphisms in OXTR are related to altruism behaviour in the Dictator game (Israel et al., 2009). The low repeat allele in the promotor region of MAOA is associated with antisocial behaviour and heightened activity in the amygdala and ventromedial prefrontal cortex activity in a face recognition task (Buckholtz & Meyer-Lindenberg, 2008). The length of a polymorphism repeat region (5HTTPLR) in the SLC6AF gene has been linked to BOLD activity in the amygdala in response to emotional stimuli, with possession of the short allele associated with heightened amygdala activation to emotional stimuli (Munafò, Brown, & Hariri, 2008).

Finally, the high heritability of neurodevelopmental disorders that affect social cognitive development speaks to the contribution of genetics to social cognition. Progress in genetic techniques have led to the discovery of multiple genetic risk factors for neurodevelopmental disorders, including common low-risk genetic variants, rare moderate-risk copy number variants, and larger chromosomal deletions (Abrahams & Geschwind, 2008; Gejman, Sanders, & Duan, 2010). The role of such genetic risk factors on behaviour and neural circuitry has been explored further in genetically modified mice. These studies have provided further support for the role of genetics in social behaviour, with many genetically modified

mice displaying disrupted social behaviour (Born et al., 2015b; Bozdagi et al., 2010; Dachtler et al., 2014; Goorden et al., 2007; Grayton et al., 2013; Jamain et al., 2008; Katayama et al., 2016; Moy et al., 2009; Peñagarikano et al., 2011b; Sato et al., 2012; Won et al., 2012b). The social phenotype displayed in these mouse models will be further described in section 6.3.1).

In chapters 2 and 3, I investigated the behaviour and electrophysiology of mice carrying a homozygous microdeletion in *Nrxn1α*. Heterozygote microdeletions in the human form of this gene (NRXN1α) are risk factors for autism and schizophrenia (Kim et al., 2008; Kirov et al., 2009; Rujescu et al., 2009), while homozygous microdeletions in this gene cause a more severe Pitt-Hopkins-like syndrome (Zweier et al., 2009). I found that homozygous microdeletions in *Nrxn1α* cause profound changes in social behaviour in mice, including changes in sociability and aggression. My results lend further support for the role of genetics in social cognition. Specifically, my results provide support for a role of the NRXN1 gene in social cognition. These results are in agreement with previous rodent studies investigating microdeletions in this gene (Grayton et al., 2013; Twining et al., 2017), as well as human studies investigating mutations in NRXN1α; in humans, deletions in NRXN1α cause changes in social behaviour that are similar to those observed in rodent models, including disinhibition and heightened aggression (Viñas-Jornet et al., 2014).

6.2.2 Environmental influences on social cognition

Experience also plays a substantial role in social cognitive development. Early life experiences shape future attachment patterns (Belsky, Houts, & Fearon, 2010). Severe neglect in early life leads to difficulties with social communication similar to those seen in autism spectrum disorders, as well as social disinhibition (Beckett et al., 2006; O'Connor et al., 1999). Experience is also important for social development during adolescence, a period during which individuals display greater sensitivity to social situations and place greater emphasis on interactions with peers (Blakemore & Mills, 2014).

Rodent studies have provided further insights into the role of experience on social behaviour. These studies have revealed that social experience during a "sensitive" period of juvenile rodent development (P21 - P36) plays a particularly important role in shaping future social behaviour. Rats that are socially isolated and prevented from engaging in play during this period are less competent social partners in adulthood, and display a range of impairments in social behaviour including heightened aggression, altered social interactions and difficulties integrating into new cage hierarchies (Potegal & Einon, 1989; Pellis & McKenna, 1995; Hol et al., 1999; van den Berg et al., 1999).

The contribution of the environment to social cognitive development is supported in my thesis. In chapters 4 and 5, I investigated the effect of changes in environment during the rodent "play" period on social development and orbitofrontal circuitry. Specifically, I explored the effect of tactile deprivation by whisker trimming during the "play" period on later social interaction. My results indicate that alterations in the sensory environment encountered by rats during the "play" period lead to changes in social interaction that outlast the sensory deprivation period. The implications of these findings are discussed further in section 6.2.3.1.

In summary, genetics and environment both play an important contribution to social development. These influences are likely to interact (e.g. through epigenetic mechanisms). This complex interplay between genetic and environmental factors, together with the prolonged time window during which experience shapes social learning, is likely to contribute to the vulnerability of social cognition to insults encountered during development.

6.2.3 "Building blocks" of social cognitive development

Social skills develop slowly across childhood and adolescence. The trajectory of social cognitive development has been well-characterised (see chapter 1.2.1). Whilst some features

of social behaviour are present from birth (e.g. preference for looking at faces), other facets, such as Theory of Mind, develop more slowly.

It is unclear to what extent social development relies on the normal acquisition of more basic social and non-social skills at younger ages. According to "cascade" models, simple social and non-social abilities (e.g. sensory perception or cognitive functions) act as a "building block" for the acquisition of more complex social skills. The role of development itself in shaping the trajectory of typical and atypical social cognition has been emphasised by "neuroconstructivist" accounts of neurodevelopment (Johnson et al., 2009; Karmiloff-Smith, 1998, 2009). However, the extent to which such "cascade" model are supported experimentally is unclear. Indeed, other researchers have that different domains of social cognition may be acquired separately and argued that there is some degree of independence between different social domains (Happé et al., 2017).

6.2.3.1 Sensory perception

Multiple lines of evidence suggest that sensory perception may influence social cognitive development. Sensory cues such as facial expressions, body posture and movement carry social meaning and guide our social interactions (Clarke et al., 2005; Coulson, 2004; Ekman & Oster, 1979). Perceptual abilities also develop in early infancy, ahead of social cognitive development. In addition, many children with neurodevelopmental disorders display sensory symptoms that correlate with social impairment (Hilton et al., 2007; Hilton et al., 2010; Liss et al., 2006; Watson et al., 2011).

In chapter 4, I investigated the effect of experiencing diminished sensory input on rodent social behaviour during a sensitive period of social development. I found that depriving rodents of whisker input during a sensitive period of social development led to changes in social play that persisted after the period of whisker deprivation. This result provides support

for the notion that sensory perception can influence social development, and indicates that sensory perception may act as a "building block" for social cognition.

Multiple studies have found a positive correlation between sensory symptoms and social cognitive impairments (Hilton et al., 2007; Hilton et al., 2010; Liss et al., 2006; Watson et al., 2011). Some rodent studies also provide support for this idea. For example, conditional expression of genetic risk factors for syndromic autism spectrum disorders restricted to sensory cells in the skin has been found to be sufficient to generate social impairments in mice (Orefice et al., 2016). Moreover, neonatal whisker trimming has been previously reported to reduce social approach (Soumiya et al., 2016). However, trimming whiskers from birth disrupts the development of whisker sensory inputs and results in compensatory changes in tactile function and neural circuitry. Therefore, in my experiments, I reduced whisker sensory input after the somatosensory system had developed. Whisker trimming has the further advantages that it is non-traumatic and reversible. This enabled me to reduce whisker sensory inputs for brief time periods. Thus, my results extend these findings by teasing apart the direct effects of impoverished sensory perception on the ability to engage in social interaction from its secondary effects on the acquisition of social behaviours itself.

Touch may play a particularly important role in shaping social cognitive development. Touch is one of the first perceptual domains to develop, and is the one of the first sensory domains used for parent-infant communication (Barnett, 2005). In addition, individuals with neurodevelopmental disorders often display particularly pronounced sensory symptoms in the domain of touch. These somatosensory symptoms share the strongest correlation with social symptoms (Hilton et al., 2010).

What role might touch play in social development? Human social touch is rewarding and carries affective meaning (Dunbar, 2010). This is also true in rodents; touch is the main

sensory domain contributing to the pleasurable properties of play (Kummer et al., 2011). Hence, social touch may contribute to the development social motivation. The partner-dependent effects of whisker trimming on play that I observed in chapter 4 appear to support this claim. In my experiments, developmental whisker trimming did not prevent rodents from performing play behaviours altogether, but appeared to alter the motivation to engage in play. Hence, touch may play a role in shaping the motivation to interact with others. These results raise the interesting possibility that tactile symptoms contribute to altered sociability in individuals with neurodevelopmental disorders. This idea merits further investigation, but is supported by the relationship between somatosensory hyper-reactivity and social avoidance in autism spectrum disorders (Hilton et al., 2007).

In addition, in chapter 5 I investigated the effect of whisker deprivation, and the subsequent effects on juvenile play development, on the development of excitatory circuitry of the "social brain". Since changes in sensory experience affected the development of juvenile play, I hypothesised that this might be accompanied by changes in neural circuitry in the orbitofrontal cortex, for the following reasons: Firstly, the orbitofrontal cortex is important for normal rodent play behaviours, and contributes to valuation of reward (Bell et al., 2006). Secondly, changes in orbitofrontal excitatory circuitry accompany play development (Bell et al., 2010). Thirdly, the orbitofrontal cortex receives sensory input from somatosensory cortex (Romanksi et al., 1999). Hence, it is possible that the absence of normal input from somatosensory cortices could lead to changes in circuitry in the orbitofrontal cortex to compensate for the lack of input, such as a weakening of somatosensory inputs at the expense of other sensory input.

My results indicated that whisker deprivation during the juvenile play period did not lead to changes in excitatory neural circuitry in the orbitofrontal cortex in adult animals. However,

the relationship between sensory input and activity in the orbitofrontal cortex is unknown. There are multiple stages of sensory processing between the barrel cortex and the orbitofrontal cortex; if sensory manipulations at this time period have weak effects at earlier stages of somatosensory processing, such as in secondary somatosensory cortex, then one may not expect significant changes in circuitry at the level of the orbitofrontal cortex. In order to explore this notion in more depth, it would be necessary to better understand the relationship between sensory experience and orbitofrontal cortex neural activity *in vivo*. For example, this could be explored by recording *in vivo* activity in the orbitofrontal cortex during the development of play behaviours, in animals with and without whisker deprivation.

6.2.3.2 Non-social cognitive development

Cognition is another domain that might influence social development. Cognitive abilities correlate with some social abilities, such as theory of mind. However, there have been questions raised with regards to direction of causality. Cognitive deficits are also observed in individuals with neurodevelopmental disorders, and have been proposed to play a "core" role in other symptoms by some researchers.

In chapter 2, I investigated whether social cognitive impairments were accompanied by cognitive deficits in a genetically modified mouse line carrying a microdeletion in *Nrxn1α*, which is a risk factor for schizophrenia and autism (Kim et al., 2008; Kirov et al., 2009; Rujescu et al., 2009). I found that impaired social cognition was not accompanied by deficits in short or long-term memory, or executive function/problem-solving.

The presence of impaired social behaviour in the absence of cognitive deficits may suggest that the molecular and neural mechanisms underlying social and cognitive impairments are partially dissociable. This is supported by some studies investigating the genetics of autism

spectrum disorder symptoms; these studies have indicated that separate clusters of genes may contribute to distinct symptoms (Ronald, Happe & Plomin, 2005; Ronald et al., 2006).

However, some caution must be applied in drawing conclusions from these results. First, microdeletions in neurexin-1α have been associated with cognitive impairment; cognitive deficits were observed in rats carrying a homozygous mutation in *Nrxn1α* (Esclassan, Francois, Phillips, Loomis, & Gilmour, 2015), and humans carrying the microdeletion often display cognitive impairments and/or intellectual disability, although it is possible that this is mediated by other genetic or environmental factors (Stefansson et al., 2013).

Second, apparent dissociations should be interpreted carefully, particularly in the case of development. Double dissociations can provide compelling evidence for two processes being caused by distinct mechanisms. However, dissociations are harder to interpret during development, since there may be heightened compensatory mechanisms, and behaviours can appear "spared" whilst having been acquired through non-normative routes (Karmiloff-Smith et al., 2003a). There are also additional issues with interpreting dissociations in rodent models. Rodent tasks often lack specificity in assessing behaviours or cognitive domains. This is particularly true for social tasks. This makes it difficult to draw any strong conclusions.

6.3 *Nrxn1α* KO mice as a model of impaired social cognition

Understanding the cellular and molecular mechanisms underlying impaired social cognition in neurodevelopmental disorders relies on having adequate animal models in which invasive techniques can be undertaken. This is difficult, because human social behaviours are highly complex, and certain features of human social cognition may be uniquely human (Rebecca Saxe, 2006). However, mice and rats are social species that engage in high levels of social interaction, suggesting that they may be used to model aspects of impaired social behaviour.

In chapter 2, I investigated the behavioural phenotype of *Nrxn1α* KO to assess their validity as a model of impaired social behaviour. Previous research has indicated that these mice display abnormal social behaviour (Grayton et al., 2013). My results replicated those of Grayton et al 2013 and indicated that these mice display robust impairments in social behaviour.

There are a number of reasons that make *Nrxn1α* KO mice an interesting model for impaired social behaviour. First, the impaired social behaviour observed in these mice is robust. This is important, as altered social behaviour is not always consistently seen in mouse models of neurodevelopmental disorders. For example, findings with regards to social behaviour in Shank 1 KO mice have been inconsistent (Sungur, Schwarting, & Wöhr, 2017). In addition, the impairments in social behaviour observed in these mice are fairly specific: my results indicate that these mice do not display impaired non-social cognition.

A third feature that makes *Nrxn1α* KO mice interesting is their unusual social phenotype. Impaired social behaviour has been reported in a number of other genetically modified mouse models carrying genetic risk factors for autism spectrum disorders, schizophrenia, and syndromic neurodevelopmental disorders. However, few mouse models display the same phenotype as *Nrxn1α* KO mice. The majority of these mouse lines display reduced sociability and reduced social interaction. For example, CNTNAP2 KO mice display reduced social interactions and reduced sociability and reduced USV calls (Peñagarikano et al., 2011c). Reduced social interaction and sociability has also been reported in mice carrying a heterozygous mutation in CDH8, TSC1 and TSC2, as well as mice carrying a homozygous deletion of Nrxn2 and Fmr1-/y mice (Born et al., 2015; Dachtler et al., 2014; Goorden et al., 2007; Katayama et al., 2016; Moy et al., 2009; Sato et al., 2012). Reduced sociability has also been reported in Oxtr KO mice, together with social recognition deficits, and reduced USV

calls (Sala et al., 2011). A phenotype consisting of reduced sociability and reduced ultrasonic communication has also been reported in mice carrying a homozygous mutation in Neuroligin 4, Shank 3 heterozygote mice, and Shank 2 KO mice (Bozdagi et al., 2010; Jamain et al., 2008; Won et al., 2012b).

In contrast, *Nrxn1α* KO mice display enhanced social approach, together with increased aggressive behaviour that is displayed in "inappropriate" contexts, for example, during interaction with juvenile mice (Grayton et al., 2013). In addition, *Nrxn1α* KO rats display impaired observational learning (Twining et al., 2017). Overall, these findings suggest that *Nrxn1α* KO mice may display a social phenotype that more closely resembles that of social disinhibition and impaired recognition of social cues. This phenotype appears fairly unique. Only one other mouse line displays a similar social phenotype to *Nrxn1α* KO mice; Dlgap2 knock-out mice also possess heightened aggression and enhanced social approach (Jiang-Xie et al., 2014).

This unusual phenotype suggests that the alterations in social behaviour seen in *Nrxn1α* KO mice differ from those reported in other mouse lines, and raises the possibility that *Nrxn1α* KO mice could be used to model other aspects of impaired social behaviour. This is important because the social cognitive impairments displayed in individuals with neurodevelopmental disorders are heterogeneous (see section 1.2.2.). Multiple facets of social cognition can be affected. Moreover, different individuals can display distinct social profiles. For example, whilst many children with autism spectrum disorders show reduced interest in social interaction, this is not the case for all children; other children make persistent attempts to engage in social interaction, but behave "oddly" in a manner that is off-putting to others (Wing & Gould, 1979). Achieving a full understanding of impaired social cognition in neurodevelopmental disorders requires modelling different facets of altered social behaviour,

and finding appropriate models to represent distinct subgroups of individuals. This will help to clarify whether differences in behavioural phenotype reflect distinct cellular and molecular aetiologies.

How valid is the *Nrxn1α* KO mouse line as a model of neurodevelopmental disease? The phenotype displayed by *Nrxn1α* KO mice suggests that they possess good face validity in the domain of altered social behaviour, since they display impaired social behaviour. In addition, the social phenotype of these mice appears similar to that observed in patients carrying the equivalent microdeletion (Viñas-Jornet et al., 2014). Moreover, the model possesses good construct validity, in that the mice carry a microdeletion that is similar to the one observed in patients. However, the mouse carries a homozygous microdeletion whereas the humans carry a heterozygous microdeletion. Therefore, there are also limits to the construct validity. The heterozygous mice do not display any behavioural phenotype. This may reflect fundamental differences between mice and humans.

The best way to determine the validity of mouse models is by evaluating predictive validity i.e. do the mice respond in predictable ways to treatment? This is difficult to do in the context of social cognition because there are few treatments for impaired social cognition. Nonetheless, it would be of interest to determine whether the *Nrxn1α* KO mice display a similar response to medication compared to patient subgroups. This would help to clarify whether *Nrxn1α* KO mice display behavioural and cellular changes that are representative of individuals with impaired social behaviour above and beyond those carrying the equivalent microdeletion in NRXN1α.

6.4 Short-term facilitation and social cognition

6.4.1 Short-term facilitation in the orbitofrontal cortex

The postsynaptic response to repeated stimulation of a synaptic input is not constant. An increase in the postsynaptic response is called facilitation whereas a decrease is termed synaptic depression (Zucker, 1989). The totality of the changes to the postsynaptic response during a train of stimuli may be referred to as either short-term synaptic dynamics or short-term synaptic plasticity. For instance, synaptic responses may facilitate early in a stimulus train, but then depress. The short-term dynamics of synaptic responses in neocortical pyramidal neurons are not fixed, but are themselves plastic (metaplasticity) and are modified by sensory experience (Cheetham et al., 2007; Finnerty et al., 1999).

In chapter 3 and 5, I investigated the short-term dynamics of orbitofrontal pyramidal layer 2/3 neurons. This is the first study examining the short-term dynamics of excitatory synapses in orbitofrontal cortex brain slices. I observed frequency-dependent short-term facilitation in the orbitofrontal cortex in response to local layer 2/3 stimulation in both rats and mice. At lower frequency stimulation (e.g. 10, 20 Hz), excitatory post-synaptic potentials in response to a pulse train displayed initial paired-pulse facilitation, followed by a decrement in the size of the EPSP. At higher frequency stimulation (40 Hz), EPSPs displayed persistent facilitation across the whole pulse train. This contrasts with findings from layer 2/3 in sensory cortex (Cheetham et al., 2007), but is similar to reports in other parts of the prefrontal cortex, which display considerable synaptic facilitation (Wang et al., 2006). Hence, like prefrontal cortical pyramidal neurons, orbitofrontal pyramidal neurons display prominent short-term facilitation.

6.4.2 Properties of prelimbic inputs to the orbitofrontal cortex

Neurons receive both short-range input from local neurons in the microcircuit, and long-range inputs from neurons in other brain regions. How post-synaptic neurons integrate local

and long-range inputs remains understudied. However, an imbalance in the integration of local and long-range inputs has been proposed as a cause of some neurodevelopmental disorders, such as autism spectrum disorders, and schizophrenia (Courchesne & Pierce, 2005; Geschwind & Flint, 2015).

The orbitofrontal cortex receives long-range inputs from a wide range of brain regions, including secondary sensory association areas, limbic and temporal regions, the thalamus/hypothalamus and other parts of the prefrontal cortex (see section 1.4.2.). How these various inputs are combined with local neuronal activity to enable the orbitofrontal cortex to perform computations for social cognition is unclear.

In chapter 3, I examined the properties of long-range inputs from prelimbic cortex onto orbitofrontal neurons. I found that, as with stimulation of short-range inputs, minimal stimulation of long-range inputs from the prelimbic cortex elicited excitatory post-synaptic responses that displayed a combination of facilitation and depression. Although not statistically significantly different, excitatory postsynaptic responses evoked by prelimbic stimulation appeared to evoke less prominent short-term facilitation than those evoked by local stimulation.

This is the first study to examine the electrophysiological properties of prelimbic inputs onto the orbitofrontal cortex. A small number of *in vivo* and *in vitro* electrophysiological studies have investigated the effects of stimulation of long-range inputs on post-synaptic potentials in the prefrontal cortex (Klavir, Prigge, Sarel, Paz, & Yizhar, 2017; Orozco-Cabal et al., 2006; Thierry et al., 2000). In some cases, this has included study of short-term synaptic dynamics. For example, a single-cell extracellular *in vivo* study in rats found that around 50% of cells in the prelimbic cortex and medial orbitofrontal cortex displayed paired-pulse facilitation in response to hippocampal stimulation (Thierry et al., 2000). In contrast, a separate *in vivo*

study using optogenetics reported short-term depression of responses in the prelimbic cortex after high frequency stimulation of inputs from the basolateral amygdala, which facilitate fear extinction (Klavir et al., 2017). However, these studies did not examine the dynamics of post-synaptic responses during longer stimulus trains. In addition, these studies did not directly compare the properties of synapses forming local connections with those of long-distance inputs originating from different brain regions.

My results provide preliminary evidence that the short-term dynamics of synapses differs as a function of the pathway in the orbitofrontal cortex. Specifically, local connections appear to display more prominent short-term facilitation. It would be of interest to assess the short-term dynamics of other sources of long-range input onto the orbitofrontal cortex and determine whether these inputs display comparable dynamics. The notion that long-range inputs display less short-term facilitation than local inputs is supported by studies in the prefrontal cortex indicating that amygdala inputs to the prefrontal cortex exhibit a greater tendency towards short-term depression than facilitation (Klavir et al., 2017).

6.4.3 Short-term dynamics of short and long-range inputs in a mouse model of autism and schizophrenia

Multiple lines of evidence indicate that autism and schizophrenia are characterised by synaptic dysfunction. A number of studies have reported changes in the number of dendritic spines in the brains of individuals with autism and schizophrenia, as well as changes in expression of synaptic proteins and in glutamate levels (Aldred, Moore, Fitzgerald, & Waring, 2003a; Harrison & Eastwood, 1998; Purcell, Jeon, Zimmerman, Blue, & Pevsner, 2001b; Shinohe et al., 2006). Many of the genes implicated in the aetiology of autism and schizophrenia are genes involved in synapse formation and function (Alarcón et al., 2008; Arking et al., 2008; Costas, 2015; Durand et al., 2007b; Fromer et al., 2014; Gauthier et al.,

2009, 2010, 2011; Jamain et al., 2003; Kim et al., 2008; Kirov et al., 2009; Laumonnier et al., 2004; Peykov et al., 2015; Rujescu et al., 2009; Strauss et al., 2006; Vaags et al., 2012).

A few different accounts of the deficits in synaptic function that may contribute to schizophrenia and autism have been proposed. One suggestion is that these conditions are characterised by excitatory-inhibitory imbalance (Rubenstein & Merzenich, 2003). Others have focussed more specifically on synapse number; some have suggested that these conditions are characterised by an excess, and reduction in the number of synapses in autism and schizophrenia respectively (Courchesne & Pierce, 2005). One recent theory of synaptic dysfunction in autism and schizophrenia proposes that these conditions are caused by an imbalance in local and long-range connectivity. Specifically, researchers have suggested that autism spectrum disorder is caused by overconnectivity of local circuits at the expense of long-range connectivity (Courchesne & Pierce, 2005). This idea has been explored through human neuroimaging studies. A number of neuroimaging studies have provided evidence consistent with this theory, with reports of reduced functional connectivity between brain regions and white matter tract abnormalities (Frazier & Hardan, 2009; Just et al., 2004; Kana et al., 2007; Koshino et al., 2005; Villalobos et al., 2005; Welchew et al., 2005). However, human neuroimaging studies lack the resolution to investigate local connectivity directly, and therefore, are limited in their ability to test this theory.

In chapter 3, I investigated the proposal that neurodevelopmental disorders are characterised by imbalances in local and long-range connectivity. I examined the effect of stimulation of local and long-range inputs from prelimbic cortex onto orbitofrontal cortex in mice carrying a homozygous microdeletion in *Nrxn1α*, a genetic risk factor for autism and schizophrenia. I found that *Nrxn1α* KO mice display reduced short-term facilitation of excitatory post-

synaptic responses in both local and long-range pathways, in the absence of other deficits in excitatory synaptic transmission.

My findings indicate that deficits in short-term facilitation may contribute to the pathophysiology of autism and schizophrenia in at least some individuals. Studies of other genetically modified mouse models also provide support for this claim. Deficits in short-term facilitation have been observed in a number of mouse models carrying genetic risk factors for schizophrenia (see Crabtree & Gogos, 2014 for review). Mice carrying the 22q11.2 microdeletion, which accounts for 1-2% of sporadic schizophrenia, display impaired short-term facilitation at synapses from hippocampus onto layer 5 medial prefrontal cortex synapses (Drew et al., 2011). Mice carrying DISC1 mutation that recapitulates the consequences of the human chromosomal translocation have reduced paired-pulse facilitation at mossy fibre inputs onto CA3 neurons (Kvajo et al., 2011a). A shift from short-term facilitation towards short-term depression has also been reported in MeCP2 null mice (Moretti & Zoghbi, 2006; Weng, McLeod, Bailey, & Cobb, 2011). In humans, mutations in MeCP2 cause most cases of Rett syndrome. Hence, it is possible that impaired short-term facilitation contributes to the pathophysiology of certain neurodevelopmental disorders. However, this is unlikely to be the only contributing factor. Other impairments in synapse function, development and neuronal migration have also been observed in mouse models of neurodevelopmental disorders. The pathophysiology of abnormal neurodevelopment is likely to be complex and vary across individuals according to their unique genetic and environmental aetiology.

My findings support the notion that excitatory synaptic function is impaired in neurodevelopmental disorders. However, my results do not support the theory that there is an excess of local connectivity at the expense of long-range connectivity. Instead, my results

suggest that both long-range and local connections are abnormal. This is consistent with more recent neuroimaging studies of autism spectrum disorders that report reduced functional connectivity in both long and short-range pathways (Khan et al., 2013). The finding that local stimulation evoked more short-term facilitation than long-range stimulation suggests that if anything, the balance of responses is shifted towards long-range inputs in *Nrxn1α* KO mice.

The pathway-dependency of synaptic changes has been investigated in small number of other rodent models of neurodevelopmental disorders. In one study, knock-down of Mef2c in a sparse population of somatosensory neurons led to reduced local synaptic connectivity and enhanced excitatory input from contralateral somatosensory cortex (Rajkovich et al., 2017). It is important to note that neurodevelopmental disorders such as autism spectrum disorders are heterogeneous conditions, both in terms of aetiology and symptoms. As such, it is highly possible that the nature of the circuit disruptions varies across individuals. Multiple circuit abnormalities may lead to similar behavioural outcomes.

The original hypotheses of long and short-range abnormalities concentrated on the idea of hyper- and hypo-connectivity i.e. overproduction or underproduction of synapses (Courchesne & Pierce, 2005). However, there are other ways in which the connections between neurons can be altered functionally, including changes in synaptic strength, timing and dynamics. In this study, no evidence was found for hyper- or hypo-connectivity of short or long-range projections, as both mini frequency, and evoked EPSP amplitude were similar in *Nrxn1α* WT and KO mice. Instead, changes were found in the short-term dynamics of excitatory responses. This highlights the point that pathway abnormalities do not necessarily involve changes in synapse number, and that other aspects of synaptic transmission should be studied. It is important to note that imaging studies possess limited ability to delve into the nature of abnormalities in neuronal circuitry.

Finally, my findings may provide some insights into why circuit abnormalities in neurodevelopmental disorders may be more pronounced in some circuits than others; even if gene abnormalities affect all brain regions, the differing properties of particular circuit pathways may make them more vulnerable to particular genetic abnormalities. In the case of *Nrxn1α* KO mice, circuits or synapses that display more short-term facilitation are more likely to be affected by the microdeletion.

6.4.4 Short-term facilitation in neural circuits

How might impaired short-term dynamics contribute to changes in behaviour seen in neurodevelopmental disorders? One way to begin to address this is to consider the effects of short-term dynamics on neural activity.

The short-term synaptic dynamics carry information about the recent history of synaptic activity on a pathway. The short-term synaptic dynamics give prominence to aspects of the temporal information in the firing pattern of the presynaptic neuron (Fortune & Rose, 2002; Fuhrmann et al., 2002). Electrophysiological experiments combined with computer simulations indicate that short-term depression accounts for low-pass filtering (Chance, Nelson, & Abbott, 1998; Rose & Fortune, 1999) and detection of changes in the rate of input firing (Abbott, Varela, Sen, & Nelson, 1997). In contrast, facilitating synapses act as high-pass filters that preferentially transmit information from burst firing neurons (Lisman, 1997; Wang, 1999). This burst activity is seen in multiple cortical areas (Connors & Gutnick, 1990; Gray & McCormick, 1996) but is particularly observed in frontal regions (Degenetais, Thierry, Glowinski, & Gioanni, 2002), and can be elicited by neuromodulator release. For example, acetylcholine promotes burst firing in thalamic reticular neurons (McCormick & Prince, 1986). Therefore, loss of short-term facilitation may reduce responsivity to neuromodulators and to burst firing. The combination of short-term depression and facilitation at different frequencies enables low-frequency stimulation to be properly

processed in the presence of high frequency ‘noise’ whilst also enabling higher frequency bursts to be processed. Hence, these dynamics may enable better signal-to-noise differentiation (Fortune & Rose, 2002).

In addition, short-term dynamics have been proposed to play a role in temporal processing, and in controlling the timing of neural responses. Computer simulations indicate that short-term changes in the strength of EPSPs and IPSPs can be used to detect temporal features of stimuli, such as their duration and order in a sequence. Moreover, short-term synaptic plasticity can evoke changes in the phase of post-synaptic responses (Chance et al., 1998; McDonnell & Graham, 2017). Specifically, short-term depression shifts the peak amplitude phase of responses forward, whereas short-term facilitation shifts the peak phase amplitude backwards. It has been suggested that changes in timing caused by short-term dynamics could be enough to compensate for synaptic transmission delays (McDonnell & Graham, 2017).

Finally, there is evidence to suggest that short-term dynamics play a role in controlling the balance of excitatory and inhibitory activity. In rat neocortex, sustained high-frequency stimulation *in vivo* leads to stronger depression in excitatory synapses than inhibitory synapses. The relative impact of inhibitory synapses increases at higher-frequency stimulation. Hence, differences in the synaptic dynamics at excitatory and inhibitory synapses may contribute to controlling and adjusting excitatory-inhibitory balance (Galarreta & Hestrin, 1998).

6.4.5 The role of short-term facilitation on behaviour

The roles of short-term facilitation at the behavioural level remain unclear. One theory proposes that short-term facilitation plays a role in working memory (Mongillo et al., 2008). According to the synaptic theory of working memory, increased residual calcium at pre-synaptic terminals enables memories to be transiently held in the absence of delay period

sustained activity by facilitating memory reactivation at these synapses. This has been proposed as an alternative to accounts of working memory that suggest that persistent increases in neuronal firing across a delay period resulting from intrinsic characteristics or reverberative activity maintains working memory (Xiao-Jing Wang, 2001). Maintenance of memory by short-term facilitation has been suggested to account for the fact that increases in firing in single neurons are not always persistent across the whole delay period (Rainer & Miller, 2002). The fact that short-term facilitation is particularly prominent in prefrontal cortical neurons, which are thought to be critical for working memory, has been taken as indirect support for this theory (Thomson, 1997; Wang et al., 2006). Simulation studies indicate that short-term facilitation could act to stabilise working memory traces (Itskov, Hansel, & Tsodyks, 2011).

However, direct support for this theory is lacking. There are some reports of genetic mutant mouse lines displaying both impaired short-term facilitation in the prefrontal cortex, and cognitive deficits including impaired working memory. For example, mice carrying a mutation in DISC-1, a risk factor for schizophrenia, display both reduced paired-pulse facilitation and cognitive deficits, although these have not always been replicated (Kvajo et al., 2008; Kvajo et al., 2011b; Nilsson et al., 2016) In addition, dysbindin mutant mice and Dcgr8 heterozygous mutant mice display reduced paired-pulse facilitation and working memory deficits (Fénelon et al., 2011b; Jentsch et al., 2009). Nonetheless, other mouse lines carrying risk factors for neurodevelopmental disorders have displayed deficits in short-term facilitation in the absence of working memory deficits (Born et al., 2015b).

In this thesis, mice carrying a homozygous deletion in *Nrxn1α*, a risk factor for numerous neurodevelopmental disorders, displayed impaired facilitation but no deficits in cognition. This corroborates previous behavioural investigations of this mouse line (Grayton et al.,

2013). Hence, my results do not support the theory that short-term synaptic facilitation is central to working memory. Other mechanisms such as recurrent reverberating activity or intrinsic persistent firing may be sufficient to maintain working memory.

Instead, the findings from my thesis suggest a role of short-term synaptic facilitation in regulating components of social behaviour. Whilst a specific role in social behaviour has not been previously proposed, a number of recent studies indicate that short-term facilitation may be required for intact social behaviour. Orexin/ataxin-3-transgenic (AT) mice display both deficits in long-term social memory and reduced hippocampal paired-pulse facilitation (Yang et al 2013). *Nrxn1α* KO rats display reduced paired-pulse facilitation in the medial amygdala, together with social fear learning deficits (Twining et al., 2017). Multiple other genetic mutant lines carrying risk factors for neurodevelopmental disorders display both reduced facilitation and impaired social behaviours (Crabtree & Gogos, 2014; Born et al., 2015).

Why might impaired short-term facilitation particularly affect social behaviour? One possible reason is that some of the brain regions that display most pronounced short-term facilitation play a role in social behaviour. Indeed, short-term facilitation is particularly pronounced in the prefrontal cortex (Wang et al., 2006), and my results indicate that this is also true in the orbitofrontal cortex. Since the prefrontal and orbitofrontal cortex display pronounced facilitation, functions subserved by these regions, such as social cognition, may be particularly affected by deficits in facilitation. Neuromodulatory influences may also be a factor contributing to the susceptibility of social cognition to impairments in short-term facilitation. Neuromodulators can alter the short-term dynamics of synapses. The prefrontal and orbitofrontal cortex receive very dense neuromodulatory input. These neuromodulators have also been linked to social cognition.

The orbitofrontal cortex receives projections from diverse brain regions. These long-range inputs are important for social functions of the orbitofrontal cortex and must be adequately integrated with local input. Since short-term facilitation plays a role in information filtering and temporal processing of neural activity, it is possible that impaired short-term facilitation disrupts integration of local and long-range inputs in the orbitofrontal cortex. We do not know how the orbitofrontal cortex integrates local and long-range inputs in the healthy brain. This should be explored in order to understand how reduced facilitation might affect integration of inputs in the orbitofrontal cortex. This could be examined by injecting retrogradely transported viruses that carry a payload encoding Channelrhodopsin into the orbitofrontal cortex to enable light-activated stimulation of distinct inputs.

6.4.6 Short-term facilitation during development

The synaptic dynamics present at synapses changes across development. A number of studies indicate that short-term facilitation emerges during post-natal development of neural circuitry. In rat sensorimotor cortex, excitatory synapses exhibit a "developmental switch" in short-term dynamics; pyramidal neurons display a greater tendency towards short-term depression at P14, which switches to weak facilitation at P28 (Reyes & Sakmann, 1999). A similar switch has been observed in layer 5 prefrontal cortical neurons, which exhibit paired-pulse depression at P7 that turns into paired-pulse facilitation by P21 (Zhang, 2003).

A similar developmental switch may occur in the orbitofrontal cortex. However, the developmental profile of short-term synaptic dynamics has not yet been investigated in the orbitofrontal cortex. Rodent studies indicate that post-natal maturation of the orbitofrontal cortex supports social cognitive development (Bell et al., 2010a). It is possible that changes in short-term synaptic dynamics are one of the mechanisms by which the orbitofrontal cortex supports social cognitive development. Future electrophysiological research should

concentrate on characterising the changes in excitatory and inhibitory circuitry that occur in the orbitofrontal cortex during sensitive periods of social development.

6.5 Conclusions

The objective of this thesis was to investigate the factors contributing to abnormal social behaviour in neurodevelopmental disorders, and to explore the role of excitatory circuitry in the orbitofrontal cortex in these abnormal social behaviours. My results indicated that social impairments in the *Nrxn1α* mouse model of autism and schizophrenia are accompanied by a deficit in short-term facilitation in layer 2/3 pyramidal orbitofrontal neurons. This deficit in short-term facilitation affects both local and long-range circuitry, and may impair integration of inputs in the orbitofrontal cortex. This result indicates that altered excitatory circuitry in the orbitofrontal cortex may contribute to social symptoms in neurodevelopmental disorders, and suggests a role of short-term facilitation in social cognition.

In addition, I found that experiencing diminished somatosensory input during a sensitive period of social development led to long-lasting changes in social behaviour in rodents that were not accompanied by altered excitatory circuitry in the orbitofrontal cortex. These results suggest that abnormalities in sensory perception may contribute to deficits in social cognition in individuals with neurodevelopmental disorders.

References

Abbott, L. F., Varela, J. A., Sen, K., & Nelson, S. B. (1997). Synaptic Depression and Cortical Gain Control. *Science, 275*(5297), 221–224. https://doi.org/10.1126/science.275.5297.221

Abrahams, B. S., & Geschwind, D. H. (2008). Advances in autism genetics: on the threshold of a new neurobiology. *Nature Reviews Genetics, 9*(5), 341–355. https://doi.org/10.1038/nrg2346

Adamson, A., O'Hare, A., & Graham, C. (2006). Impairments in Sensory Modulation in Children with Autistic Spectrum Disorder. *British Journal of Occupational Therapy, 69*(8), 357–364. https://doi.org/10.1177/030802260606900803

Adolphs, R. (1999). Social cognition and the human brain. *Trends in Cognitive Sciences, 3*(12), 469–479. https://doi.org/10.1016/S1364-6613(99)01399-6

Adolphs, R. (2001). The neurobiology of social cognition. *Current Opinion in Neurobiology, 11*(2), 231–239. https://doi.org/10.1016/S0959-4388(00)00202-6

Adolphs, R. (2006). How do we know the minds of others? Domain-specificity, simulation, and enactive social cognition. *Brain Research, 1079*(1), 25–35. https://doi.org/10.1016/J.BRAINRES.2005.12.127

Adolphs, R. (2009). The social brain: neural basis of social knowledge. *Annual Review of Psychology*, 60, 693-716.

Adolphs, R. (2010). What does the amygdala contribute to social cognition? *Annals of the New York Academy of Sciences, 1191*(1), 42–61. https://doi.org/10.1111/j.1749-6632.2010.05445.x

Adolphs, R., Sears, L., & Piven, J. (2001). Abnormal Processing of Social Information from Faces in Autism. *Journal of Cognitive Neuroscience*, *13*(2), 232–240. https://doi.org/10.1162/089892901564289

Adolphs, R., Tranel, D., Damasio, H., & Damasio, A. R. (1995). Fear and the Human Amygdala. *The Journal of Neuroscience*, *75*(g), 5879–5891.

Agha, Z., Iqbal, Z., Kleefstra, T., Zweier, C., Pfundt, R., Qamar, R., … Willemsen, M. H. (2015). A de novo microdeletion in NRXN1 in a Dutch patient with mild intellectual disability, microcephaly and gonadal dysgenesis. *Genetics Research*, *97*, e19. https://doi.org/10.1017/S001667231500021X

Ago, Y., Araki, R., Tanaka, T., Sasaga, A., Nishiyama, S., Takuma, K., & Matsuda, T. (2013). Role of Social Encounter-Induced Activation of Prefrontal Serotonergic Systems in the Abnormal Behaviors of Isolation-Reared Mice. *Neuropsychopharmacology*, *38*(8), 1535–1547. https://doi.org/10.1038/npp.2013.52

Ainsworth, M. S. (1979). Infant–mother attachment. *American Psychologist*, *34*(10), 932–937. https://doi.org/10.1037/0003-066X.34.10.932

Alarcón, M., Abrahams, B. S., Stone, J. L., Duvall, J. A., Perederiy, J. V., Bomar, J. M., … Geschwind, D. H. (2008). Linkage, Association, and Gene-Expression Analyses Identify CNTNAP2 as an Autism-Susceptibility Gene. *The American Journal of Human Genetics*, *82*(1), 150–159. https://doi.org/10.1016/J.AJHG.2007.09.005

Albieri, G., Barnes, S. J., de Celis Alonso, B., Cheetham, C. E. J., Edwards, C. E., Lowe, A. S., … Finnerty, G. T. (2015). Rapid Bidirectional Reorganization of Cortical Microcircuits. *Cerebral Cortex*, *25*(9), 3025–3035. https://doi.org/10.1093/cercor/bhu098

Aldred, S., Moore, K. M., Fitzgerald, M., & Waring, R. H. (2003a). Plasma Amino Acid Levels in Children with Autism and Their Families. *Journal of Autism and Developmental Disorders*, *33*(1), 93–97. https://doi.org/10.1023/A:1022238706604

Alexander, A. L., Lee, J. E., Lazar, M., Boudos, R., DuBray, M. B., Oakes, T. R., … Lainhart, J. E. (2007). Diffusion tensor imaging of the corpus callosum in Autism. *NeuroImage*, *34*(1), 61–73. https://doi.org/10.1016/J.NEUROIMAGE.2006.08.032

Amaral, D. G., & Price, J. L. (1984). Amygdalo-cortical projections in the monkey (Macaca fascicularis). *The Journal of Comparative Neurology*, *230*(4), 465–496. https://doi.org/10.1002/cne.902300402

Amodio, D. M., & Frith, C. D. (2006). Meeting of minds: the medial frontal cortex and social cognition. *Nature Reviews Neuroscience*, *7*(4), 268–277. https://doi.org/10.1038/nrn1884

Anderson, S. W., Damasio, H., Tranel, D., & Damasio, A. R. (2000). Long-Term Sequelae of Prefrontal Cortex Damage Acquired in Early Childhood. *Developmental Neuropsychology*, *18*(3), 281–296. https://doi.org/10.1207/S1532694202Anderson

Anisfeld, M. (1979). Interpreting "imitative" responses in early infancy. *Science*, *205*(4402), 214–215. https://doi.org/10.1126/science.451593

Anisfeld, M. (1996). Only Tongue Protrusion Modeling Is Matched by Neonates. *Developmental Review*, *16*(2), 149–161. https://doi.org/10.1006/DREV.1996.0006

Anisfeld, M., Turkewitz, G., Rose, S. A., Rosenberg, F. R., Sheiber, F. J., Couturier-Fagan, D. A., … Sommer, I. (2001). No Compelling Evidence That Newborns Imitate Oral Gestures. *Infancy*, *2*(1), 111–122. https://doi.org/10.1207/S15327078IN0201_7

Anney, R., Klei, L., Pinto, D., Almeida, J., Bacchelli, E., Baird, G., … Devlin, B. (2012). Individual common variants exert weak effects on the risk for autism spectrum disorders. *Human Molecular Genetics*, *21*(21), 4781–4792. https://doi.org/10.1093/hmg/dds301

Aoto, J., Martinelli, D. C., Malenka, R. C., Tabuchi, K., & Südhof, T. C. (2013). Presynaptic Neurexin-3 Alternative Splicing trans-Synaptically Controls Postsynaptic AMPA Receptor Trafficking. *Cell*, *154*(1), 75–88. https://doi.org/10.1016/j.cell.2013.05.060

American Psychiatric Association. (2013). Diagnostic and statistical manual of mental disorders (5th ed.). Arlington, VA: American Psychiatric Publishing.

Arakawa, H., Blanchard, D. C., Arakawa, K., & Dunlap, C. (2008). Scent marking behavior as an odorant communication in mice. *Neuroscience & Biobehavioral Reviews*, *32*(7), 1236–1248. https://doi.org/10.1016/J.NEUBIOREV.2008.05.012

Arakawa, H., & Erzurumlu, R. S. (2015). Role of whiskers in sensorimotor development of C57BL/6 mice. *Behavioural Brain Research*, *287*, 146–155. https://doi.org/10.1016/j.bbr.2015.03.040

Arking, D. E., Cutler, D. J., Brune, C. W., Teslovich, T. M., West, K., Ikeda, M., … Chakravarti, A. (2008). A Common Genetic Variant in the Neurexin Superfamily Member CNTNAP2 Increases Familial Risk of Autism. *The American Journal of Human Genetics*, *82*(1), 160–164. https://doi.org/10.1016/J.AJHG.2007.09.015

Asaka, Y., Jugloff, D. G. M., Zhang, L., Eubanks, J. H., & Fitzsimonds, R. M. (2006). Hippocampal synaptic plasticity is impaired in the Mecp2-null mouse model of Rett syndrome. *Neurobiology of Disease*, *21*(1), 217–227. https://doi.org/10.1016/j.nbd.2005.07.005

Ashwin, C., Chapman, E., Colle, L., & Baron-Cohen, S. (2006). Impaired recognition of negative basic emotions in autism: A test of the amygdala theory. *Social Neuroscience*, *1*(3–4), 349–363. https://doi.org/10.1080/17470910601040772

Atluri, P. P., & Regehr, W. G. (1996). Determinants of the Time Course of Facilitation at the Granule Cell to Purkinje Cell Synapse. *Journal of Neuroscience*, *16*(18).

Bachevalier, J., & Loveland, K. A. (2006). The orbitofrontal–amygdala circuit and self-regulation of social–emotional behavior in autism. *Neuroscience & Biobehavioral Reviews*, *30*(1), 97–117. https://doi.org/10.1016/J.NEUBIOREV.2005.07.002

Bachner-Melman, R., Zohar, A. H., Bacon-Shnoor, N., Elizur, Y., Nemanov, L., Gritsenko, I., & Ebstein, R. P. (2005). Link Between Vasopressin Receptor *AVPR1A* Promoter Region Microsatellites and Measures of Social Behavior in Humans. *Journal of Individual Differences*, *26*(1), 2–10. https://doi.org/10.1027/1614-0001.26.1.2

Bailey, A., Le Couteur, A., Gottesman, I., Bolton, P., Simonoff, E., Yuzda, E., & Rutter, M. (1995). Autism as a strongly genetic disorder: evidence from a British twin study. *Psychological Medicine*, *25*(1), 63. https://doi.org/10.1017/S0033291700028099

Baillargeon, R., Scott, R. M., & He, Z. (2010). False-belief understanding in infants. *Trends in Cognitive Sciences*, *14*(3), 110–118. https://doi.org/10.1016/J.TICS.2009.12.006

Baird, G., Simonoff, E., Pickles, A., Chandler, S., Loucas, T., Meldrum, D., & Charman, T. (2006). Prevalence of disorders of the autism spectrum in a population cohort of children in South Thames: the Special Needs and Autism Project (SNAP). *The Lancet*, *368*(9531), 210–215. https://doi.org/10.1016/S0140-6736(06)69041-7

Bannerman, D. M., Niewoehner, B., Lyon, L., Romberg, C., Schmitt, W. B., Taylor, A., …

Rawlins, J. N. P. (2008). NMDA receptor subunit NR2A is required for rapidly acquired spatial working memory but not incremental spatial reference memory. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *28*(14), 3623–30. https://doi.org/10.1523/JNEUROSCI.3639-07.2008

Baranek, G. T., David, F. J., Poe, M. D., Stone, W. L., & Watson, L. R. (2006). Sensory Experiences Questionnaire: discriminating sensory features in young children with autism, developmental delays, and typical development. *Journal of Child Psychology and Psychiatry*, *47*(6), 591–601. https://doi.org/10.1111/j.1469-7610.2005.01546.x

Barbas, H. (1988). Anatomic organization of basoventral and mediodorsal visual recipient prefrontal regions in the rhesus monkey. *The Journal of Comparative Neurology*, *276*(3), 313–342. https://doi.org/10.1002/cne.902760302

Barbas, H. (1993). Organization of cortical afferent input to orbitofrontal areas in the rhesus monkey. *Neuroscience*, *56*(4), 841–864.

Barbas, H., & Pandya, D. N. (1989). Architecture and intrinsic connections of the prefrontal cortex in the rhesus monkey. *The Journal of Comparative Neurology*, *286*(3), 353–375. https://doi.org/10.1002/cne.902860306

Barfield, R. J., Auerbach, P., Geyer, L. A., & Mcintosh, T. K. (1979). Ultrasonic Vocalizations in Rat Sexual Behavior. *American Zoologist*, *19*(2), 469–480. https://doi.org/10.1093/icb/19.2.469

Barnea-Goraly, N., Kwon, H., Menon, V., Eliez, S., Lotspeich, L., & Reiss, A. L. (2004). White matter structure in autism: preliminary evidence from diffusion tensor imaging. *Biological Psychiatry*, *55*(3), 323–326. https://doi.org/10.1016/J.BIOPSYCH.2003.10.022

Barnea-Goraly, N., Menon, V., Eckert, M., Tamm, L., Bammer, R., Karchemskiy, A., … Reiss, A. L. (2005). White Matter Development During Childhood and Adolescence: A Cross-sectional Diffusion Tensor Imaging Study. *Cerebral Cortex*, *15*(12), 1848–1854. https://doi.org/10.1093/cercor/bhi062

Barnes, S. J., Cheetham, C. E., Liu, Y., Bennett, S. H., Albieri, G., Jorstad, A. A., … Finnerty, G. T. (2015). Delayed and Temporally Imprecise Neurotransmission in Reorganizing Cortical Microcircuits. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *35*(24), 9024–37. https://doi.org/10.1523/JNEUROSCI.4583-14.2015

Barnes, S. J., & Finnerty, G. T. (2010). Sensory Experience and Cortical Rewiring. *The Neuroscientist*, *16*(2), 186–198. https://doi.org/10.1177/1073858409343961

Barnett, L. (2005). Keep in touch: The importance of touch in infant development. *Infant Observation*, *8*(2), 115–123. https://doi.org/10.1080/13698030500171530

Baron-Cohen, S. (1989). Perceptual role taking and protodeclarative pointing in autism. *British Journal of Developmental Psychology*, *7*(2), 113–127. https://doi.org/10.1111/j.2044-835X.1989.tb00793.x

Baron-Cohen, S., Jolliffe, T., Mortimore, C., & Robertson, M. (1997). Another Advanced Test of Theory of Mind: Evidence from Very High Functioning Adults with Autism or Asperger Syndrome. *Journal of Child Psychology and Psychiatry*, *38*(7), 813–822. https://doi.org/10.1111/j.1469-7610.1997.tb01599.x

Baron-Cohen, S., Leslie, A. M., & Frith, U. (1985). Does the autistic child have a "theory of mind"? *Cognition*, *21*(1), 37–46. https://doi.org/10.1016/0010-0277(85)90022-8

Baron-Cohen, S., O'Riordan, M., Stone, V., Jones, R., & Plaisted, K. (1999). Recognition of Faux Pas by Normally Developing Children and Children with Asperger Syndrome or High-Functioning Autism. *Journal of Autism and Developmental Disorders*, *29*(5), 407–418. https://doi.org/10.1023/A:1023035012436

Baron-Cohen, S., Ring, H., Moriarty, J., Schmitz, B., Costa, D., & Ell, P. (1994). Recognition of mental state terms. Clinical findings in children with autism and a functional neuroimaging study of normal adults. *The British Journal of Psychiatry : The Journal of Mental Science*, *165*(5), 640–9. https://doi.org/10.1192/BJP.165.5.640

Baron-Cohen, S., & Wheelwright, S. (2003). The Friendship Questionnaire: An Investigation of Adults with Asperger Syndrome or High-Functioning Autism, and Normal Sex Differences. *Journal of Autism and Developmental Disorders*, *33*(5). Retrieved from http://docs.autismresearchcentre.com/papers/2003_BCandSW_FQ.pdf

Baron-Cohen, S., Wheelwright, S., Hill, J., Raste, Y., & Plumb, I. (2001). The "Reading the Mind in the Eyes" Test revised version: a study with normal adults, and adults with Asperger syndrome or high-functioning autism. *Journal of Child Psychology and Psychiatry, and Allied Disciplines*, *42*(2), 241–51. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11280420

Barrash, J., Tranel, D., & Anderson, S. W. (2000). Acquired Personality Disturbances Associated With Bilateral Damage to the Ventromedial Prefrontal Region. *Developmental Neuropsychology*, *18*(3), 355–381. https://doi.org/10.1207/S1532694205Barrash

Bartak, L., & Rutter, M. (1976). Differences between mentally retarded and normally intelligent autistic children. *Journal of Autism and Childhood Schizophrenia*, *6*(2), 109–

120. https://doi.org/10.1007/BF01538054

Barton, J. J. S., Press, D. Z., Keenan, J. P., & O'Connor, M. (2002). Lesions of the fusiform face area impair perception of facial configuration in prosopagnosia. *Neurology*, *58*(1), 71–8. https://doi.org/10.1212/WNL.58.1.71

Bas-Hoogendam, J. M., van Steenbergen, H., Kreuk, T., van der Wee, N. J. A., & Westenberg, P. M. (2017). How embarrassing! The behavioral and neural correlates of processing social norm violations. *PLOS ONE*, *12*(4), e0176326. https://doi.org/10.1371/journal.pone.0176326

Batki, A., Baron-Cohen, S., Wheelwright, S., Connellan, J., & Ahluwalia, J. (2000). Is there an innate gaze module? Evidence from human neonates. *Infant Behavior and Development*, *23*(2), 223–229. https://doi.org/10.1016/S0163-6383(01)00037-6

Baylis, L. L., Rolls, E. T., & Baylis, G. C. (1995). Afferent connections of the caudolateral orbitofrontal cortex taste area of the primate. *Neuroscience*, *64*(3), 801–812. https://doi.org/10.1016/0306-4522(94)00449-F

Bechara, A., Damasio, A. R., Damasio, H., & Anderson, S. W. (1994). Insensitivity to future consequences following damage to human prefrontal cortex. *Cognition*, *50*(1–3), 7–15. https://doi.org/10.1016/0010-0277(94)90018-3

Beckett, C., Maughan, B., Rutter, M., Castle, J., Colvert, E., Groothues, C., … Sonuga-Barke, E. J. S. (2006). Do the Effects of Early Severe Deprivation on Cognition Persist Into Early Adolescence? Findings From the English and Romanian Adoptees Study. *Child Development*, *77*(3), 696–711. https://doi.org/10.1111/j.1467-8624.2006.00898.x

Beer, J. S., Heerey, E. A., Keltner, D., Scabini, D., & Knight, R. T. (2003). The regulatory

function of self-conscious emotion: Insights from patients with orbitofrontal damage. *Journal of Personality and Social Psychology*, *85*(4), 594–604. https://doi.org/10.1037/0022-3514.85.4.594

Beer, J. S., & Ochsner, K. N. (2006). Social cognition: A multi level analysis. *Brain Research*, *1079*(1), 98–105. https://doi.org/10.1016/J.BRAINRES.2006.01.002

Behrens, T. E. J., Hunt, L. T., Woolrich, M. W., & Rushworth, M. F. S. (2008). Associative learning of social value. *Nature*, *456*(7219), 245–249. https://doi.org/10.1038/nature07538

Bell, H. C., Pellis, S. M., & Kolb, B. (2010a). Juvenile peer play experience and the development of the orbitofrontal and medial prefrontal cortices. *Behavioural Brain Research*, *207*(1), 7–13. https://doi.org/10.1016/j.bbr.2009.09.029

Bell, H. C., Pellis, S. M., & Kolb, B. (2010b). Juvenile peer play experience and the development of the orbitofrontal and medial prefrontal cortices. *Behavioural Brain Research*, *207*(1), 7–13. https://doi.org/10.1016/j.bbr.2009.09.029

Bellugi, U., & George, M. I. S. (2000). Linking cognitive neuroscience and molecular genetics: new perspectives from Williams syndrome. MIT Press.

Belsky, J., Houts, R. M., & Fearon, R. M. P. (2010). Infant Attachment Security and the Timing of Puberty. *Psychological Science*, *21*(9), 1195–1201. https://doi.org/10.1177/0956797610379867

Ben-Ami Bartal, I., Decety, J., & Mason, P. (2011). Empathy and pro-social behavior in rats. *Science (New York, N.Y.)*, *334*(6061), 1427–30. https://doi.org/10.1126/science.1210789

Ben-Sasson, A., Hen, L., Fluss, R., Cermak, S. A., Engel-Yeger, B., & Gal, E. (2009). A

Meta-Analysis of Sensory Modulation Symptoms in Individuals with Autism Spectrum Disorders. *Journal of Autism and Developmental Disorders*, *39*(1), 1–11. https://doi.org/10.1007/s10803-008-0593-3

Ben Bashat, D., Kronfeld-Duenias, V., Zachor, D. A., Ekstein, P. M., Hendler, T., Tarrasch, R., … Ben Sira, L. (2007). Accelerated maturation of white matter in young children with autism: A high b value DWI study. *NeuroImage*, *37*(1), 40–47. https://doi.org/10.1016/J.NEUROIMAGE.2007.04.060

Benes, F. M., McSparren, J., Bird, E. D., SanGiovanni, J. P., & Vincent, S. L. (1991). Deficits in small interneurons in prefrontal and cingulate cortices of schizophrenic and schizoaffective patients. *Archives of General Psychiatry*, *48*(11), 996–1001.

Berlin, H. A., Rolls, E. T., & Kischka, U. (2004). Impulsivity, time perception, emotion and reinforcement sensitivity in patients with orbitofrontal cortex lesions. *Brain*, *127*(5), 1108–1126. https://doi.org/10.1093/brain/awh135

Berthoz, S., Armony, J. L., Blair, R. J. R., & Dolan, R. J. (2002). An fMRI study of intentional and unintentional (embarrassing) violations of social norms. *Brain*, *125*(8), 1696–1708. https://doi.org/10.1093/brain/awf190

Betancur, C. (2011). Etiological heterogeneity in autism spectrum disorders: More than 100 genetic and genomic disorders and still counting. *Brain Research*, *1380*, 42–77. https://doi.org/10.1016/J.BRAINRES.2010.11.078

Beyer, F., Münte, T. F., Göttlich, M., Krämer, U. M., AJ, C., N, D., … K, Z. (2015). Orbitofrontal Cortex Reactivity to Angry Facial Expression in a Social Interaction Correlates with Aggressive Behavior. *Cerebral Cortex*, *25*(9), 3057–3063. https://doi.org/10.1093/cercor/bhu101

Biederer, T., & Südhof, T. C. (2000). Mints as adaptors. Direct binding to neurexins and recruitment of munc18. *The Journal of Biological Chemistry*, *275*(51), 39803–6. https://doi.org/10.1074/jbc.C000656200

Bielsky, I. F., Hu, S.-B., Szegda, K. L., Westphal, H., & Young, L. J. (2004). Profound Impairment in Social Recognition and Reduction in Anxiety-Like Behavior in Vasopressin V1a Receptor Knockout Mice. *Neuropsychopharmacology*, *29*(3), 483–493. https://doi.org/10.1038/sj.npp.1300360

Bird, G., Leighton, J., Press, C., & Heyes, C. (2007). Intact automatic imitation of human and robot actions in autism spectrum disorders. *Proceedings. Biological Sciences*, *274*(1628), 3027–31. https://doi.org/10.1098/rspb.2007.1019

Biro, L., Toth, M., Sipos, E., Bruzsik, B., Tulogdi, A., Bendahan, S., … Haller, J. (2017). Structural and functional alterations in the prefrontal cortex after post-weaning social isolation: relationship with species-typical and deviant aggression. *Brain Structure and Function*, *222*(4), 1861–1875. https://doi.org/10.1007/s00429-016-1312-z

Blake, R., Turner, L. M., Smoski, M. J., Pozdol, S. L., & Stone, W. L. (2003). Visual Recognition of Biological Motion is Impaired in Children With Autism. *Psychological Science*, *14*(2), 151–157. https://doi.org/10.1111/1467-9280.01434

Blakemore, S.-J. (2008). The social brain in adolescence. *Nature Reviews Neuroscience*, *9*(4), 267–277. https://doi.org/10.1038/nrn2353

Blakemore, S.-J., & Mills, K. L. (2014). Is Adolescence a Sensitive Period for Sociocultural Processing? *Annual Review of Psychology*, *65*(1), 187–207. https://doi.org/10.1146/annurev-psych-010213-115202

Blanchard, J. J., Bellack, A. S., & Mueser, K. T. (1994). Affective and social-behavioral correlates of physical and social anhedonia in schizophrenia. *Journal of Abnormal Psychology*, *103*(4), 719–728. https://doi.org/10.1037/0021-843X.103.4.719

Bobrov, E., Wolfe, J., Rao, R. P., & Brecht, M. (2014). The Representation of Social Facial Touch in Rat Barrel Cortex. *Current Biology*, *24*(1), 109–115. https://doi.org/10.1016/j.cub.2013.11.049

Bock, J., Murmu, R. P., Ferdman, N., Leshem, M., & Braun, K. (2008a). Refinement of dendritic and synaptic networks in the rodent anterior cingulate and orbitofrontal cortex: Critical impact of early and late social experience. *Developmental Neurobiology*, *68*(5), 685–695. https://doi.org/10.1002/dneu.20622

Bolivar, V. J., Walters, S. R., & Phoenix, J. L. (2007). Assessing autism-like behavior in mice: Variations in social interactions among inbred strains. *Behavioural Brain Research*, *176*(1), 21–26. https://doi.org/10.1016/J.BBR.2006.09.007

Boogert, N. J., Giraldeau, L.-A., & Lefebvre, L. (2008). Song complexity correlates with learning ability in zebra finch males. *Animal Behaviour*, *76*(5), 1735–1741. https://doi.org/10.1016/J.ANBEHAV.2008.08.009

Bora, E., Yucel, M., & Pantelis, C. (2009). Theory of mind impairment in schizophrenia: Meta-analysis. *Schizophrenia Research*, *109*(1–3), 1–9. https://doi.org/10.1016/J.SCHRES.2008.12.020

Born, G., Grayton, H. M., Langhorst, H., Dudanova, I., Rohlmann, A., Woodward, B. W., … Missler, M. (2015a). Genetic targeting of NRXN2 in mice unveils role in excitatory cortical synapse function and social behaviors. *Frontiers in Synaptic Neuroscience*, *7*, 3. https://doi.org/10.3389/fnsyn.2015.00003

Bourgeron, T. (2009). A synaptic trek to autism. *Current Opinion in Neurobiology*, *19*(2), 231–234. https://doi.org/10.1016/J.CONB.2009.06.003

Bozdagi, O., Sakurai, T., Papapetrou, D., Wang, X., Dickstein, D. L., Takahashi, N., … Buxbaum, J. D. (2010). Haploinsufficiency of the autism-associated Shank3 gene leads to deficits in synaptic function, social interaction, and social communication. *Molecular Autism*, *1*(1), 15. https://doi.org/10.1186/2040-2392-1-15

Branchi, I., Santucci, D., & Alleva, E. (2001). Ultrasonic vocalisation emitted by infant rodents: a tool for assessment of neurobehavioural development. *Behavioural Brain Research*, *125*(1–2), 49–56. https://doi.org/10.1016/S0166-4328(01)00277-7

Branchi, I., Santucci, D., & Alleva, E. (2001). Ultrasonic vocalisation emitted by infant rodents: a tool for assessment of neurobehavioural development. *Behavioural Brain Research*, *125*(1–2), 49–56.

Brauer, J., Xiao, Y., Poulain, T., Friederici, A. D., & Schirmer, A. (2016). Frequency of Maternal Touch Predicts Resting Activity and Connectivity of the Developing Social Brain. *Cerebral Cortex (New York, N.Y. : 1991)*, *26*(8), 3544–52. https://doi.org/10.1093/cercor/bhw137

Breton, F., Planté, A., Legauffre, C., Morel, N., Adès, J., Gorwood, P., … Dubertret, C. (2011). The executive control of attention differentiates patients with schizophrenia, their first-degree relatives and healthy controls. *Neuropsychologia*, *49*(2), 203–208. https://doi.org/10.1016/j.neuropsychologia.2010.11.019

Brothers, L. (1996). Brain mechanisms of social cognition. *Journal of Psychopharmacology*, *10*(1), 2–8. https://doi.org/10.1177/026988119601000102

Brown, R. Z. (1953). Social Behavior, Reproduction, and Population Changes in the House Mouse (Mus musculus L.). *Ecological Monographs*, *23*(3), 217–240. https://doi.org/10.2307/1943592

Brudzynski, S. M. (2013). Ethotransmission: communication of emotional states through ultrasonic vocalization in rats. *Current Opinion in Neurobiology*, *23*(3), 310–317. https://doi.org/10.1016/J.CONB.2013.01.014

Brune, M. (2005). "Theory of Mind" in Schizophrenia: A Review of the Literature. *Schizophrenia Bulletin*, *31*(1), 21–42. https://doi.org/10.1093/schbul/sbi002

Buckholtz, J. W., & Meyer-Lindenberg, A. (2008). MAOA and the neurogenetic architecture of human aggression. *Trends in Neurosciences*, *31*(3), 120–129. https://doi.org/10.1016/J.TINS.2007.12.006

Bundy, A. C., Shia, S., Qi, L., & Miller, L. J. (n.d.). How does sensory processing dysfunction affect play? *The American Journal of Occupational Therapy : Official Publication of the American Occupational Therapy Association*, *61*(2), 201–8.

Burgdorf, J., Kroes, R. A., Moskal, J. R., Pfaus, J. G., Brudzynski, S. M., & Panksepp, J. (2008). Ultrasonic vocalizations of rats (Rattus norvegicus) during mating, play, and aggression: Behavioral concomitants, relationship to reward, and self-administration of playback.. *Journal of Comparative Psychology*, *122*(4), 357–367. https://doi.org/10.1037/a0012889

Burgdorf, J., & Panksepp, J. (2001). Tickling induces reward in adolescent rats. *Physiology & Behavior*, *72*(1–2), 167–173. https://doi.org/10.1016/S0031-9384(00)00411-X

Burke, C. J., Kisko, T. M., Swiftwolfe, H., Pellis, S. M., & Euston, D. R. (2017). Specific 50-

kHz vocalizations are tightly linked to particular types of behavior in juvenile rats anticipating play. *PloS One*, *12*(5), e0175841. https://doi.org/10.1371/journal.pone.0175841

Burke, K. A., Franz, T. M., Miller, D. N., & Schoenbaum, G. (2008). The role of the orbitofrontal cortex in the pursuit of happiness and more specific rewards. *Nature*, *454*(7202), 340–4. https://doi.org/10.1038/nature06993

Burks, J. D., Conner, A. K., Bonney, P. A., Glenn, C. A., Baker, C. M., Boettcher, L. B., … Sughrue, M. E. (2017). Anatomy and white matter connections of the orbitofrontal gyrus. *Journal of Neurosurgery*, 1–8. https://doi.org/10.3171/2017.3.JNS162070

Burnett, A. C., Reutens, D. C., & Wood, A. G. (2010). Social cognition in Turner's Syndrome. *Journal of Clinical Neuroscience*, *17*(3), 283–286. https://doi.org/10.1016/J.JOCN.2009.09.006

Bush, G., Luu, P., & Posner, M. I. (2000). Cognitive and emotional influences in anterior cingulate cortex. *Trends in Cognitive Sciences*, *4*(6), 215–222. https://doi.org/10.1016/S1364-6613(00)01483-2

Buttelmann, D., Carpenter, M., & Tomasello, M. (2009). Eighteen-month-old infants show false belief understanding in an active helping paradigm. *Cognition*, *112*(2), 337–342. https://doi.org/10.1016/J.COGNITION.2009.05.006

Butter, C. M., McDonald, J. A., & Snyder, D. R. (1969). Orality, preference behavior, and reinforcement value of nonfood object in monkeys with orbital frontal lesions. *Science (New York, N.Y.)*, *164*(3885), 1306–7. https://doi.org/10.1126/SCIENCE.164.3885.1306

Calcagnetti, D. J., & Schechter, M. D. (1992). Place conditioning reveals the rewarding

aspect of social interaction in juvenile rats. *Physiology & Behavior*, *51*(4), 667–672. https://doi.org/10.1016/0031-9384(92)90101-7

Candland, D. K., & Nagy, Z. M. (1969). The open field: some comparative data. *Annals of the New York Academy of Sciences*, *159*(3 Experimental), 831–851. https://doi.org/10.1111/j.1749-6632.1969.tb12982.x

Cao, X. (2017). Functions of synapse adhesion molecules neurexin/neuroligins and neurodevelopmental disorders. *Neuroscience Research*, *116*, 3–9. https://doi.org/10.1016/J.NEURES.2016.09.005

Carlson, S. M., Moses, L. J., & Breton, C. (2002). How specific is the relation between executive function and theory of mind? Contributions of inhibitory control and working memory. *Infant and Child Development*, *11*(2), 73–92. https://doi.org/10.1002/icd.298

Carmichael, S. T., & Price, J. L. (1995a). Limbic connections of the orbital and medial prefrontal cortex in macaque monkeys. *The Journal of Comparative Neurology*, *363*(4), 615–641. https://doi.org/10.1002/cne.903630408

Carmichael, S. T., & Price, J. L. (1995b). Sensory and premotor connections of the orbital and medial prefrontal cortex of macaque monkeys. *The Journal of Comparative Neurology*, *363*(4), 642–664. https://doi.org/10.1002/cne.903630409

Carvell, G. E., & Simons, D. J. (1996). Abnormal tactile experience early in life disrupts active touch. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *16*(8), 2750–7.

Caspi, A., McClay, J., Moffitt, T. E., Mill, J., Martin, J., Craig, I. W., … Poulton, R. (2002). Role of genotype in the cycle of violence in maltreated children. *Science (New York,*

N.Y.), *297*(5582), 851–4. https://doi.org/10.1126/science.1072290

Castelli, F., Happé, F., Frith, U., & Frith, C. (2000). Movement and Mind: A Functional Imaging Study of Perception and Interpretation of Complex Intentional Movement Patterns. *NeuroImage*, *12*(3), 314–325. https://doi.org/10.1006/NIMG.2000.0612

Cesarini, D., Johannesson, M., Lichtenstein, P., Sandewall, Ö., & Wallace, B. (2010). Genetic variation in financial decision-making. *The Journal of Finance*, 65(5), 1725-1754.

Chadman, K. K., Yang, M., & Crawley, J. N. (2009). Criteria for validating mouse models of psychiatric diseases. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*, *150B*(1), 1–11. https://doi.org/10.1002/ajmg.b.30777

Challis, C., Beck, S. G., & Berton, O. (2014). Optogenetic modulation of descending prefrontocortical inputs to the dorsal raphe bidirectionally bias socioaffective choices after social defeat. *Frontiers in Behavioral Neuroscience*, *8*, 43. https://doi.org/10.3389/fnbeh.2014.00043

Chance, F. S., Nelson, S. B., & Abbott, L. F. (1998). Synaptic Depression and the Temporal Response Characteristics of V1 Cells. *Journal of Neuroscience*, *18*(12).

Chang, C., & Ho, T. (2017). Inhibitory modulation of medial prefrontal cortical activation on lateral orbitofrontal cortex-amygdala information flow. *The Journal of Physiology*, *595*(17), 6065–6076. https://doi.org/10.1113/JP274568

Charman, T., Baron-Cohen, S., Swettenham, J., Baird, G., Cox, A., & Drew, A. (2000). Testing joint attention, imitation, and play as infancy precursors to language and theory of mind. *Cognitive Development*, *15*(4), 481–498. https://doi.org/10.1016/S0885-

2014(01)00037-5

Charman, T., Swettenham, J., Baron-Cohen, S., Cox, A., Baird, G., & Drew, A. (1997). Infants with autism: An investigation of empathy, pretend play, joint attention, and imitation. *Developmental Psychology*, *33*(5), 781–789. https://doi.org/10.1037/0012-1649.33.5.781

Chartrand, T. L., & Bargh, J. A. (1999). The chameleon effect: The perception–behavior link and social interaction. *Journal of Personality and Social Psychology*, *76*(6), 893–910. https://doi.org/10.1037/0022-3514.76.6.893

Cheetham, C. E. J., Hammond, M. S. L., Edwards, C. E. J., & Finnerty, G. T. (2007). Sensory Experience Alters Cortical Connectivity and Synaptic Function Site Specifically. *Journal of Neuroscience*, *27*(13).

Cheng, Y., Chou, K.-H., Chen, I.-Y., Fan, Y.-T., Decety, J., & Lin, C.-P. (2010). Atypical development of white matter microstructure in adolescents with autism spectrum disorders. *NeuroImage*, *50*(3), 873–882. https://doi.org/10.1016/J.NEUROIMAGE.2010.01.011

Chevallier, C., Kohls, G., Troiani, V., Brodkin, E. S., & Schultz, R. T. (2012a). The social motivation theory of autism. *Trends in Cognitive Sciences*, *16*(4), 231–239. https://doi.org/10.1016/J.TICS.2012.02.007

Chevallier, C., Kohls, G., Troiani, V., Brodkin, E. S., & Schultz, R. T. (2012b). The social motivation theory of autism. *Trends in Cognitive Sciences*, *16*(4), 231–9. https://doi.org/10.1016/j.tics.2012.02.007

Ching, M. S. L., Shen, Y., Tan, W.-H., Jeste, S. S., Morrow, E. M., Chen, X., … Wu, B.-L.

(2010). Deletions of NRXN1 (neurexin-1) predispose to a wide spectrum of developmental disorders. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*, *153B*(4), n/a-n/a. https://doi.org/10.1002/ajmg.b.31063

Choudhury, S., Blakemore, S.-J., & Charman, T. (2006). Social cognitive development during adolescence. *Social Cognitive and Affective Neuroscience*, *1*(3), 165–74. https://doi.org/10.1093/scan/nsl024

Ciaramelli, E., Muccioli, M., Làdavas, E., & di Pellegrino, G. (2007a). Selective deficit in personal moral judgment following damage to ventromedial prefrontal cortex. *Social Cognitive and Affective Neuroscience*, *2*(2), 84–92. https://doi.org/10.1093/scan/nsm001

Ciaramelli, E., Muccioli, M., Làdavas, E., & di Pellegrino, G. (2007b). Selective deficit in personal moral judgment following damage to ventromedial prefrontal cortex. *Social Cognitive and Affective Neuroscience*, *2*(2), 84–92. https://doi.org/10.1093/scan/nsm001

Cicerone, K. D., & Tanenbaum, L. N. (1997). Disturbance of social cognition after traumatic orbitofrontal brain injury. *Archives of Clinical Neuropsychology*, *12*(2), 173–188. https://doi.org/10.1093/arclin/12.2.173

Cirulli, E. T., & Goldstein, D. B. (2010). Uncovering the roles of rare variants in common disease through whole-genome sequencing. *Nature Reviews Genetics*, *11*(6), 415–425. https://doi.org/10.1038/nrg2779

Clarke, T. J., Bradshaw, M. F., Field, D. T., Hampson, S. E., & Rose, D. (2005). The Perception of Emotion from Body Movement in Point-Light Displays of Interpersonal Dialogue. *Perception*, *34*(10), 1171–1180. https://doi.org/10.1068/p5203

Cleare, A. J., & Bond, A. J. (1995). The effect of tryptophan depletion and enhancement on

subjective and behavioural aggression in normal male subjects. *Psychopharmacology*, *118*(1), 72–81. https://doi.org/10.1007/BF02245252

Colvert, E., Tick, B., McEwen, F., Stewart, C., Curran, S. R., Woodhouse, E., … Bolton, P. (2015). Heritability of Autism Spectrum Disorder in a UK Population-Based Twin Sample. *JAMA Psychiatry*, *72*(5), 415. https://doi.org/10.1001/jamapsychiatry.2014.3028

Condé, F., Maire-lepoivre, E., Audinat, E., & Crépel, F. (1995). Afferent connections of the medial frontal cortex of the rat. II. Cortical and subcortical afferents. *Journal of Comparative Neurology*, *352*(4), 567–593. https://doi.org/10.1002/cne.903520407

Connors, B. W., & Gutnick, M. J. (1990). Intrinsic firing patterns of diverse neocortical neurons. *Trends in Neurosciences*, *13*(3), 99–104. https://doi.org/10.1016/0166-2236(90)90185-D

Cook, J. L., & Bird, G. (2012). Atypical Social Modulation of Imitation in Autism Spectrum Conditions. *Journal of Autism and Developmental Disorders*, *42*(6), 1045–1051. https://doi.org/10.1007/s10803-011-1341-7

Cook, R., Press, C., Dickinson, A., & Heyes, C. (2010). Acquisition of automatic imitation is sensitive to sensorimotor contingency. *Journal of Experimental Psychology: Human Perception and Performance*, *36*(4), 840–852. https://doi.org/10.1037/a0019256

Cook Jr, E. H., & Scherer, S. W. (2008). Copy-number variations associated with neuropsychiatric conditions. *Nature*, *455*(7215), 919–923. https://doi.org/10.1038/nature07458

Corbett, B. A., Muscatello, R. A., & Blain, S. D. (2016). Impact of Sensory Sensitivity on

Physiological Stress Response and Novel Peer Interaction in Children with and without Autism Spectrum Disorder. *Frontiers in Neuroscience*, *10*, 278. https://doi.org/10.3389/fnins.2016.00278

Corbetta, M., & Shulman, G. L. (2002). Control of goal-directed and stimulus-driven attention in the brain. *Nature Reviews Neuroscience*, *3*(3), 215–229. https://doi.org/10.1038/nrn755

Cornish, K., Burack, J. A., Rahman, A., Munir, F., Russo, N., & Grant, C. (2005). Theory of mind deficits in children with fragile X syndrome. *Journal of Intellectual Disability Research*, *49*(5), 372–378. https://doi.org/10.1111/J.1365-2788.2005.00678.X

Costas, J. (2015). The role of SHANK2 rare variants in schizophrenia susceptibility. *Molecular Psychiatry*, *20*(12), 1486–1486. https://doi.org/10.1038/mp.2015.119

Coulson, M. (2004). Attributing Emotion to Static Body Postures: Recognition Accuracy, Confusions, and Viewpoint Dependence. *Journal of Nonverbal Behavior*, *28*(2), 117–139. https://doi.org/10.1023/B:JONB.0000023655.25550.be

Courchesne, E., & Pierce, K. (2005). Why the frontal cortex in autism might be talking only to itself: local over-connectivity but long-distance disconnection. *Current Opinion in Neurobiology*, *15*(2), 225–230. https://doi.org/10.1016/J.CONB.2005.03.001

Couture, S. M., Penn, D. L., & Roberts, D. L. (2006). The Functional Significance of Social Cognition in Schizophrenia: A Review. *Schizophrenia Bulletin*, *32*(Supplement 1), S44–S63. https://doi.org/10.1093/schbul/sbl029

Crabtree, G. W., & Gogos, J. A. (2014). Synaptic plasticity, neural circuits, and the emerging role of altered short-term information processing in schizophrenia. *Frontiers in Synaptic*

Neuroscience, *6*, 28. https://doi.org/10.3389/fnsyn.2014.00028

Crabtree, G. W., Sun, Z., Kvajo, M., Broek, J. A. C., Fénelon, K., McKellar, H., … Gogos, J. A. (2017). Alteration of Neuronal Excitability and Short-Term Synaptic Plasticity in the Prefrontal Cortex of a Mouse Model of Mental Illness. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *37*(15), 4158–4180. https://doi.org/10.1523/JNEUROSCI.4345-15.2017

Crawley, J. N., Chen, T., Puri, A., Washburn, R., Sullivan, T. L., Hill, J. M., … Young, W. S. (2007). Social approach behaviors in oxytocin knockout mice: Comparison of two independent lines tested in different laboratory environments. *Neuropeptides*, *41*(3), 145–163. https://doi.org/10.1016/J.NPEP.2007.02.002

Crespi, B., Stead, P., & Elliot, M. (2010). Evolution in health and medicine Sackler colloquium: Comparative genomics of autism and schizophrenia. *Proceedings of the National Academy of Sciences of the United States of America*, *107 Suppl 1*(suppl 1), 1736–41. https://doi.org/10.1073/pnas.0906080106

Crinella, F. M., & Yu, J. (1995). Brain mechanisms in problem solving and intelligence: A replication and extension. *Intelligence*, *21*(2), 225–246. https://doi.org/10.1016/0160-2896(95)90027-6

Critchley, H. D., & Rolls, E. T. (1996a). Hunger and satiety modify the responses of olfactory and visual neurons in the primate orbitofrontal cortex. *Journal of Neurophysiology*, *75*(4), 1673–86.

Critchley, H. D., & Rolls, E. T. (1996b). Olfactory neuronal responses in the primate orbitofrontal cortex: analysis in an olfactory discrimination task. *Journal of Neurophysiology*, *75*(4), 1659–72.

Cushman, F., Sheketoff, R., Wharton, S., & Carey, S. (2013). The development of intent-based moral judgment. *Cognition*, *127*(1), 6–21. https://doi.org/10.1016/J.COGNITION.2012.11.008

Dabell, M. P., Rosenfeld, J. A., Bader, P., Escobar, L. F., El-Khechen, D., Vallee, S. E., … Shaffer, L. G. (2013). Investigation of *NRXN1* deletions: Clinical and molecular characterization. *American Journal of Medical Genetics Part A*, *161*(4), 717–731. https://doi.org/10.1002/ajmg.a.35780

Dachtler, J., Glasper, J., Cohen, R. N., Ivorra, J. L., Swiffen, D. J., Jackson, A. J., … Clapcote, S. J. (2014). Deletion of α-neurexin II results in autism-related behaviors in mice. *Translational Psychiatry*, *4*(11), e484. https://doi.org/10.1038/tp.2014.123

Dani, V. S., Chang, Q., Maffei, A., Turrigiano, G. G., Jaenisch, R., & Nelson, S. B. (2005). Reduced cortical activity due to a shift in the balance between excitation and inhibition in a mouse model of Rett syndrome. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(35), 12560–5. https://doi.org/10.1073/pnas.0506071102

Dani, V. S., & Nelson, S. B. (2009). Intact long-term potentiation but reduced connectivity between neocortical layer 5 pyramidal neurons in a mouse model of Rett syndrome. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *29*(36), 11263–70. https://doi.org/10.1523/JNEUROSCI.1019-09.2009

Davies, M., Udwin, O., & Howlin, P. (1998). Adults with Williams syndrome. Preliminary study of social, emotional and behavioural difficulties. *The British Journal of Psychiatry : The Journal of Mental Science*, *172*(3), 273–6. https://doi.org/10.1192/BJP.172.3.273

Daw, N. W., & Wyatt, H. J. (1976). Kittens reared in a unidirectional environment: evidence for a critical period. *The Journal of Physiology*, *257*(1), 155–170. https://doi.org/10.1113/jphysiol.1976.sp011361

Dawson, G., Toth, K., Abbott, R., Osterling, J., Munson, J., Estes, A., & Liaw, J. (2004). Early Social Attention Impairments in Autism: Social Orienting, Joint Attention, and Attention to Distress. *Developmental Psychology*, *40*(2), 271–283. https://doi.org/10.1037/0012-1649.40.2.271

Dawson, G., & Watling, R. (2000). Interventions to Facilitate Auditory, Visual, and Motor Integration in Autism: A Review of the Evidence. *Journal of Autism and Developmental Disorders*, *30*(5), 415–421. https://doi.org/10.1023/A:1005547422749

De Almeida, R. M. M., Rosa, M. M., Santos, D. M., Saft, D. M., Benini, Q., & Miczek, K. A. (2006). 5-HT1B receptors, ventral orbitofrontal cortex, and aggressive behavior in mice. *Psychopharmacology*, *185*(4), 441–450. https://doi.org/10.1007/s00213-006-0333-3

De Bruin, J. P. C. (1990). Orbital prefrontal cortex, dopamine, and social-agonistic behavior of male long evans rats. *Aggressive Behavior*, *16*(3–4), 231–248. https://doi.org/10.1002/1098-2337(1990)16:3/4<231::AID-AB2480160309>3.0.CO;2-1

De Bruin, J. P. C., Van Oyen, H. G. M., & Van De Poll, N. (1983). Behavioural changes following lesions of the orbital prefrontal cortex in male rats. *Behavioural Brain Research*, *10*(2–3), 209–232. https://doi.org/10.1016/0166-4328(83)90032-3

De Paola, V., Holtmaat, A., Knott, G., Song, S., Wilbrecht, L., Caroni, P., & Svoboda, K. (2006). Cell Type-Specific Structural Plasticity of Axonal Branches and Boutons in the Adult Neocortex. *Neuron*, *49*(6), 861–875. https://doi.org/10.1016/j.neuron.2006.02.017

Degenetais, E., Thierry, A.-M., Glowinski, J., & Gioanni, Y. (2002). Electrophysiological Properties of Pyramidal Neurons in the Rat Prefrontal Cortex: An In Vivo Intracellular Recording Study. *Cerebral Cortex*, *12*(1), 1–16. https://doi.org/10.1093/cercor/12.1.1

del Castillo, J., & Katz, B. (1954). Quantal components of the end-plate potential. *The Journal of Physiology*, *124*(3), 560–573. https://doi.org/10.1113/jphysiol.1954.sp005129

Dere, E., Huston, J. P., & De Souza Silva, M. A. (2007). The pharmacology, neuroanatomy and neurogenetics of one-trial object recognition in rodents. *Neuroscience & Biobehavioral Reviews*, *31*(5), 673–704. https://doi.org/10.1016/J.NEUBIOREV.2007.01.005

Desai, N. S., Cudmore, R. H., Nelson, S. B., & Turrigiano, G. G. (2002). Critical periods for experience-dependent synaptic scaling in visual cortex. *Nature Neuroscience*, *5*(8), 783–789. https://doi.org/10.1038/nn878

Dimitropoulos, A., Ho, A. Y., Klaiman, C., Koenig, K., & Schultz, R. T. (2009). A Comparison of Behavioral and Emotional Characteristics in Children with Autism, Prader-Willi Syndrome, and Williams Syndrome. *Journal of Mental Health Research in Intellectual Disabilities*, *2*(3), 220–243. https://doi.org/10.1080/19315860903052204

Dodd, H. F., & Porter, M. A. (2010). I see happy people: Attention bias towards happy but not angry facial expressions in Williams syndrome. *Cognitive Neuropsychiatry*, *15*(6), 549–567. https://doi.org/10.1080/13546801003737157

Downey, L. E., Mahoney, C. J., Buckley, A. H., Golden, H. L., Henley, S. M., Schmitz, N., … Warren, J. D. (2015). White matter tract signatures of impaired social cognition in frontotemporal lobar degeneration. *NeuroImage. Clinical*, *8*, 640–51. https://doi.org/10.1016/j.nicl.2015.06.005

Doyle, T. F., Bellugi, U., Korenberg, J. R., & Graham, J. (2004). Everybody in the world is my friend? hypersociability in young children with Williams syndrome. *American Journal of Medical Genetics*, *124A*(3), 263–273. https://doi.org/10.1002/ajmg.a.20416

Drew, L. J., Stark, K. L., Fénelon, K., Karayiorgou, M., MacDermott, A. B., & Gogos, J. A. (2011). Evidence for altered hippocampal function in a mouse model of the human 22q11.2 microdeletion. *Molecular and Cellular Neuroscience*, *47*(4), 293–305. https://doi.org/10.1016/J.MCN.2011.05.008

Dumontheil, I., Apperly, I. A., & Blakemore, S.-J. (2010). Online usage of theory of mind continues to develop in late adolescence. *Developmental Science*, *13*(2), 331–338. https://doi.org/10.1111/j.1467-7687.2009.00888.x

Dumontheil, I., Houlton, R., Christoff, K., & Blakemore, S.-J. (2010). Development of relational reasoning during adolescence. *Developmental Science*, *13*(6), F15–F24. https://doi.org/10.1111/j.1467-7687.2010.01014.x

Dunbar, R. I. M. (2010). The social role of touch in humans and primates: Behavioural function and neurobiological mechanisms. *Neuroscience & Biobehavioral Reviews*, *34*(2), 260–268. https://doi.org/10.1016/J.NEUBIOREV.2008.07.001

Durand, C. M., Betancur, C., Boeckers, T. M., Bockmann, J., Chaste, P., Fauchereau, F., … Bourgeron, T. (2007). Mutations in the gene encoding the synaptic scaffolding protein SHANK3 are associated with autism spectrum disorders. *Nature Genetics*, *39*(1), 25–27. https://doi.org/10.1038/ng1933

Einfeld, S. L., Tonge, B. J., & Florio, T. (1997). Behavioral and Emotional Disturbance in Individuals With Williams Syndrome. *American Journal on Mental Retardation*, *102*(1), 45. https://doi.org/10.1352/0895-8017(1997)102<0045:BAEDII>2.0.CO;2

Einfeld, S. L., Tonge, B. J., & Rees, V. W. (2001). Longitudinal Course of Behavioral and Emotional Problems in Williams Syndrome. *American Journal on Mental Retardation*, *106*(1), 73. https://doi.org/10.1352/0895-8017(2001)106<0073:LCOBAE>2.0.CO;2

Einfeld, S., Tonge, B., Turner, G., Parmenter, T., & Smith, A. (1999). Longitudinal course of behavioural and emotional problems of young persons with Prader-Willi, Fragile X, Williams and Down syndromes. *Journal of Intellectual & Developmental Disability*, *24*(4), 349–354. https://doi.org/10.1080/13668259900034111

Eisenberg, N. (2000). Emotion, Regulation, and Moral Development. *Annual Review of Psychology*, *51*(1), 665–697. https://doi.org/10.1146/annurev.psych.51.1.665

Eisenberger, N. I., & Cole, S. W. (2012). Social neuroscience and health: neurophysiological mechanisms linking social ties with physical health. *Nature Neuroscience*, *15*(5), 669–674. https://doi.org/10.1038/nn.3086

Eisenberger, N. I., Lieberman, M. D., & Williams, K. D. (2003). Does rejection hurt? An FMRI study of social exclusion. *Science (New York, N.Y.)*, *302*(5643), 290–2. https://doi.org/10.1126/science.1089134

Eisner, P., Klasen, M., Wolf, D., Zerres, K., Eggermann, T., Eisert, A., … Mathiak, K. (2017). Cortico-limbic connectivity in *MAOA* -L carriers is vulnerable to acute tryptophan depletion. *Human Brain Mapping*, *38*(3), 1622–1635. https://doi.org/10.1002/hbm.23475

Ekman, P. (1992). Are there basic emotions? *Psychological Review*, *99*(3), 550–553. https://doi.org/10.1037/0033-295X.99.3.550

Ekman, P., & Oster, H. (1979). Facial Expressions of Emotion. *Annual Review of*

Psychology, *30*(1), 527–554. https://doi.org/10.1146/annurev.ps.30.020179.002523

Elison, J. T., Wolff, J. J., Heimer, D. C., Paterson, S. J., Gu, H., Hazlett, H. C., … IBIS Network. (2013). Frontolimbic neural circuitry at 6 months predicts individual differences in joint attention at 9 months. *Developmental Science*, *16*(2), 186–197. https://doi.org/10.1111/desc.12015

Elliott, R., Dolan, R. J., & Frith, C. D. (2000). Dissociable Functions in the Medial and Lateral Orbitofrontal Cortex: Evidence from Human Neuroimaging Studies. *Cerebral Cortex*, *10*(3), 308–317. https://doi.org/10.1093/cercor/10.3.308

Ellison-Wright, I., & Bullmore, E. (2009). Meta-analysis of diffusion tensor imaging studies in schizophrenia. *Schizophrenia Research*, *108*(1–3), 3–10. https://doi.org/10.1016/J.SCHRES.2008.11.021

Emerson, E. (2012). Deprivation, ethnicity and the prevalence of intellectual and developmental disabilities. *Journal of Epidemiology and Community Health*, *66*(3), 218–224. https://doi.org/10.1136/jech.2010.111773

Esclassan, F., Francois, J., Phillips, K. G., Loomis, S., & Gilmour, G. (2015). Phenotypic characterization of nonsocial behavioral impairment in neurexin 1α knockout rats. *Behavioral Neuroscience*, *129*(1), 74–85. https://doi.org/10.1037/bne0000024

Eslinger, P. J., & Damasio, A. R. (1985). Severe disturbance of higher cognition after bilateral frontal lobe ablation: patient EVR. *Neurology*, *35*(12), 1731–41. https://doi.org/10.1212/WNL.35.12.1731

Etherton, M. R., Blaiss, C. A., Powell, C. M., & Südhof, T. C. (2009). Mouse neurexin-1alpha deletion causes correlated electrophysiological and behavioral changes consistent

with cognitive impairments. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(42), 17998–8003. https://doi.org/10.1073/pnas.0910297106

Etkin, A., Egner, T., Peraza, D. M., Kandel, E. R., & Hirsch, J. (2006). Resolving Emotional Conflict: A Role for the Rostral Anterior Cingulate Cortex in Modulating Activity in the Amygdala. *Neuron*, *51*(6), 871–882. https://doi.org/10.1016/J.NEURON.2006.07.029

Fairhurst, M. T., Löken, L., & Grossmann, T. (2014). Physiological and behavioral responses reveal 9-month-old infants' sensitivity to pleasant touch. *Psychological Science*, *25*(5), 1124–31. https://doi.org/10.1177/0956797614527114

Falkner, A. L., Grosenick, L., Davidson, T. J., Deisseroth, K., & Lin, D. (2016). Hypothalamic control of male aggression-seeking behavior. *Nature Neuroscience*, *19*(4), 596–604. https://doi.org/10.1038/nn.4264

Farovik, A., Place, R. J., McKenzie, S., Porter, B., Munro, C. E., & Eichenbaum, H. (2015). Orbitofrontal cortex encodes memories within value-based schemas and represents contexts that guide memory retrieval. *Journal of Neuroscience*, 35(21), 8333-8344.

Farroni, T., Mansfield, E. M., Lai, C., & Johnson, M. H. (2003). Infants perceiving and acting on the eyes: tests of an evolutionary hypothesis. *Journal of Experimental Child Psychology*, *85*(3), 199–212.

Fatt, P., & Katz, B. (1952). Spontaneous subthreshold activity at motor nerve endings. *The Journal of Physiology*, *117*(1), 109–128. https://doi.org/10.1113/JPHYSIOL.1952.SP004735

Fedchyshyn, M. J., & Wang, L.-Y. (2007). Activity-dependent changes in temporal

components of neurotransmission at the juvenile mouse calyx of Held synapse. *The Journal of Physiology*, *581*(2), 581–602. https://doi.org/10.1113/jphysiol.2007.129833

Feinberg, I. (1982). Schizophrenia: Caused by a fault in programmed synaptic elimination during adolescence? *Journal of Psychiatric Research*, *17*(4), 319–334. https://doi.org/10.1016/0022-3956(82)90038-3

Felix-Ortiz, A. C., & Tye, K. M. (2014). Amygdala inputs to the ventral hippocampus bidirectionally modulate social behavior. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *34*(2), 586–95. https://doi.org/10.1523/JNEUROSCI.4257-13.2014

Fénelon, K., Mukai, J., Xu, B., Hsu, P.-K., Drew, L. J., Karayiorgou, M., … Gogos, J. A. (2011a). Deficiency of Dgcr8, a gene disrupted by the 22q11.2 microdeletion, results in altered short-term plasticity in the prefrontal cortex. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(11), 4447–52. https://doi.org/10.1073/pnas.1101219108

Fénelon, K., Mukai, J., Xu, B., Hsu, P.-K., Drew, L. J., Karayiorgou, M., … Gogos, J. A. (2011b). Deficiency of Dgcr8, a gene disrupted by the 22q11.2 microdeletion, results in altered short-term plasticity in the prefrontal cortex. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(11), 4447–52. https://doi.org/10.1073/pnas.1101219108

Ferdman, N., Murmu, R., Bock, J., Braun, K., & Leshem, M. (2007). Weaning age, social isolation, and gender, interact to determine adult explorative and social behavior, and dendritic and spine morphology in prefrontal cortex of rats. *Behavioural Brain Research*, *180*(2), 174–182. https://doi.org/10.1016/j.bbr.2007.03.011

Filiano, A. J., Xu, Y., Tustison, N. J., Marsh, R. L., Baker, W., Smirnov, I., … Kipnis, J. (2016). Unexpected role of interferon-γ in regulating neuronal connectivity and social behaviour. *Nature*, *535*(7612), 425–429. https://doi.org/10.1038/nature18626

Finnerty, G. T., Roberts, L. S. E., & Connors, B. W. (1999). Sensory experience modifies the short-term dynamics of neocortical synapses. *Nature*, *400*(6742), 367–371. https://doi.org/10.1038/22553

Fletcher-Watson, S., Findlay, J. M., Leekam, S. R., & Benson, V. (2008). Rapid Detection of Person Information in a Naturalistic Scene. *Perception*, *37*(4), 571–583. https://doi.org/10.1068/p5705

Foley, D. L., Eaves, L. J., Wormley, B., Silberg, J. L., Maes, H. H., Kuhn, J., & Riley, B. (2004). Childhood Adversity, Monoamine Oxidase A Genotype, and Risk for ConductDisorder. *Archives of General Psychiatry*, *61*(7), 738. https://doi.org/10.1001/archpsyc.61.7.738

Folstein, S. E., & Rosen-Sheidley, B. (2001). Genetics of austim: complex aetiology for a heterogeneous disorder. *Nature Reviews Genetics*, *2*(12), 943–955. https://doi.org/10.1038/35103559

Fombonne, E. (2003). Epidemiological surveys of autism and other pervasive developmental disorders: an update. *Journal of Autism and Developmental Disorders*, *33*(4), 365–82.

Fone, K. C. F., & Porkess, M. V. (2008). Behavioural and neurochemical effects of post-weaning social isolation in rodents—Relevance to developmental neuropsychiatric disorders. *Neuroscience & Biobehavioral Reviews*, *32*(6), 1087–1102. https://doi.org/10.1016/J.NEUBIOREV.2008.03.003

Ford, C. E., Jones, K. W., Polani, P. E., De Almeida, J. C., & Briggs, J. H. (1959). A sex-chromosome anomaly in a case of gonadal dysgenesis (Turner's syndrome). *The Lancet*, *273*(7075), 711–713. https://doi.org/10.1016/S0140-6736(59)91893-8

Fortune, E. S., & Rose, G. J. (2002). Roles for short-term synaptic plasticity in behavior. *Journal of Physiology-Paris*, *96*(5–6), 539–545. https://doi.org/10.1016/S0928-4257(03)00009-3

Foss-Feig, J. H., Tadin, D., Schauder, K. B., & Cascio, C. J. (2013). A substantial and unexpected enhancement of motion perception in autism. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *33*(19), 8243–9. https://doi.org/10.1523/JNEUROSCI.1608-12.2013

Frazier, T. W., & Hardan, A. Y. (2009). A Meta-Analysis of the Corpus Callosum in Autism. *Biological Psychiatry*, *66*(10), 935–941. https://doi.org/10.1016/J.BIOPSYCH.2009.07.022

Frerking, M., Borges, S., & Wilson, M. (1997). Are some minis multiquantal?. *Journal of Neurophysiology*, 78(3), 1293-1304.

Frith, C. (2004). Is autism a disconnection disorder? *The Lancet. Neurology*, *3*(10), 577. https://doi.org/10.1016/S1474-4422(04)00875-0

Frith, C. D. (2007). The social brain? *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *362*(1480), 671–8. https://doi.org/10.1098/rstb.2006.2003

Frith, C. D., & Corcoran, R. (1996). Exploring "theory of mind" in people with schizophrenia. *Psychological Medicine*, *26*(3), 521.

https://doi.org/10.1017/S0033291700035601

Frith, C. D., & Frith, U. (2012). Mechanisms of Social Cognition. *Annual Review of Psychology*, *63*(1), 287–313. https://doi.org/10.1146/annurev-psych-120710-100449

Fromer, M., Pocklington, A. J., Kavanagh, D. H., Williams, H. J., Dwyer, S., Gormley, P., … O'Donovan, M. C. (2014). De novo mutations in schizophrenia implicate synaptic networks. *Nature*, *506*(7487), 179–184. https://doi.org/10.1038/nature12929

Fuhrmann, G., Segev, I., Markram, H., & Tsodyks, M. (2002). Coding of temporal information by activity-dependent synapses. *Journal of Neurophysiology*, *87*(1), 140–8.

Gabbott, P. L. A., Warner, T. A., Jays, P. R. L., Salway, P., & Busby, S. J. (2005). Prefrontal cortex in the rat: Projections to subcortical autonomic, motor, and limbic centers. *The Journal of Comparative Neurology*, *492*(2), 145–177. https://doi.org/10.1002/cne.20738

Gagliardi, C., Frigerio, E., Burt, D. M., Cazzaniga, I., Perrett, D. I., & Borgatti, R. (2003). Facial expression recognition in Williams syndrome. *Neuropsychologia*, *41*(6), 733–738. https://doi.org/10.1016/S0028-3932(02)00178-1

Galarreta, M., & Hestrin, S. (1998). Frequency-dependent synaptic depression and the balance of excitation and inhibition in the neocortex. *Nature Neuroscience*, *1*(7), 587–594. https://doi.org/10.1038/2822

García-Cabezas, M. Á., & Barbas, H. (2016). Anterior Cingulate Pathways May Affect Emotions Through Orbitofrontal Cortex. *Cerebral Cortex*, *27*(10), 4891–4910. https://doi.org/10.1093/cercor/bhw284

Garey, L. J., Ong, W. Y., Patel, T. S., Kanani, M., Davis, A., Mortimer, A. M., … Hirsch, S. R. (1998). Reduced dendritic spine density on cerebral cortical pyramidal neurons in

schizophrenia. *Journal of Neurology, Neurosurgery, and Psychiatry*, *65*(4), 446–53. https://doi.org/10.1136/JNNP.65.4.446

Gauthier, I., Skudlarski, P., Gore, J. C., & Anderson, A. W. (2000). Expertise for cars and birds recruits brain areas involved in face recognition. *Nature Neuroscience*, *3*(2), 191–197. https://doi.org/10.1038/72140

Gauthier, I., Tarr, M. J., Anderson, A. W., Skudlarski, P., & Gore, J. C. (1999). Activation of the middle fusiform "face area" increases with expertise in recognizing novel objects. *Nature Neuroscience*, *2*(6), 568–573. https://doi.org/10.1038/9224

Gauthier, J., Champagne, N., Lafrenière, R. G., Xiong, L., Spiegelman, D., Brustein, E., … Gourion, D. (2010). De novo mutations in the gene encoding the synaptic scaffolding protein SHANK3 in patients ascertained for schizophrenia. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(17), 7863–8. https://doi.org/10.1073/pnas.0906232107

Gauthier, J., Siddiqui, T. J., Huashan, P., Yokomaku, D., Hamdan, F. F., Champagne, N., … Rouleau, G. A. (2011). Truncating mutations in NRXN2 and NRXN1 in autism spectrum disorders and schizophrenia. *Human Genetics*, *130*(4), 563–573. https://doi.org/10.1007/s00439-011-0975-z

Gauthier, J., Spiegelman, D., Piton, A., Lafrenière, R. G., Laurent, S., St-Onge, J., … Rouleau, G. A. (2009). Novel de novo SHANK3 mutation in autistic patients. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*, *150B*(3), 421–424. https://doi.org/10.1002/ajmg.b.30822

Gazzola, V., Spezio, M. L., Etzel, J. A., Castelli, F., Adolphs, R., & Keysers, C. (2012). Primary somatosensory cortex discriminates affective significance in social touch.

Proceedings of the National Academy of Sciences of the United States of America, *109*(25), E1657-66. https://doi.org/10.1073/pnas.1113211109

Geiger, J. R. P., & Jonas, P. (2000). Dynamic Control of Presynaptic Ca2+ Inflow by Fast-Inactivating K+ Channels in Hippocampal Mossy Fiber Boutons. *Neuron*, *28*(3), 927–939. https://doi.org/10.1016/S0896-6273(00)00164-1

Gejman, P. V, Sanders, A. R., & Duan, J. (2010). The role of genetics in the etiology of schizophrenia. *The Psychiatric Clinics of North America*, *33*(1), 35–66. https://doi.org/10.1016/j.psc.2009.12.003

Geppert, M., Khvotchev, M., Krasnoperov, V., Goda, Y., Missler, M., Hammer, R. E., … Südhof, T. C. (1998). Neurexin I alpha is a major alpha-latrotoxin receptor that cooperates in alpha-latrotoxin action. *The Journal of Biological Chemistry*, *273*(3), 1705–10. https://doi.org/10.1074/JBC.273.3.1705

Gerlai, R. (1996). Gene-targeting studies of mammalian behavior: is it the mutation or the background genotype? *Trends in Neurosciences*, *19*(5), 177–81.

Geschwind, D. H., & Flint, J. (2015). Genetics and genomics of psychiatric disease. *Science (New York, N.Y.)*, *349*(6255), 1489–94. https://doi.org/10.1126/science.aaa8954

Giedd, J. N., Blumenthal, J., Jeffries, N. O., Castellanos, F. X., Liu, H., Zijdenbos, A., … Rapoport, J. L. (1999). Brain development during childhood and adolescence: a longitudinal MRI study. *Nature Neuroscience*, *2*(10), 861–863. https://doi.org/10.1038/13158

Girgis, R. R., Minshew, N. J., Melhem, N. M., Nutche, J. J., Keshavan, M. S., & Hardan, A. Y. (2007). Volumetric alterations of the orbitofrontal cortex in autism. *Progress in*

Neuro-Psychopharmacology and Biological Psychiatry, *31*(1), 41–45. https://doi.org/10.1016/J.PNPBP.2006.06.007

Glantz, L. A., & Lewis, D. A. (2000). Decreased Dendritic Spine Density on Prefrontal Cortical Pyramidal Neurons in Schizophrenia. *Archives of General Psychiatry*, *57*(1), 65. https://doi.org/10.1001/archpsyc.57.1.65

Gogtay, N., Giedd, J. N., Lusk, L., Hayashi, K. M., Greenstein, D., Vaituzis, A. C., ... & Rapoport, J. L. (2004). Dynamic mapping of human cortical development during childhood through early adulthood. *Proceedings of the National academy of Sciences of the United States of America*, 101(21), 8174-8179.

Golan, O., Baron-Cohen, S., Hill, J. J., & Rutherford, M. D. (2007). The "Reading the Mind in the Voice" Test-Revised: A Study of Complex Emotion Recognition in Adults with and Without Autism Spectrum Conditions. *Journal of Autism and Developmental Disorders*, *37*(6), 1096–1106. https://doi.org/10.1007/s10803-006-0252-5

Gold, J. M., & Harvey, P. D. (1993). Cognitive deficits in schizophrenia. *The Psychiatric Clinics of North America*, *16*(2), 295–312.

Good, C. D., Lawrence, K., Thomas, N. S., Price, C. J., Ashburner, J., Friston, K. J., … Skuse, D. H. (2003). Dosage-sensitive X-linked locus influences the development of amygdala and orbitofrontal cortex, and fear recognition in humans. *Brain*, *126*(11), 2431–2446. https://doi.org/10.1093/brain/awg242

Goorden, S. M. I., van Woerden, G. M., van der Weerd, L., Cheadle, J. P., & Elgersma, Y. (2007). Cognitive deficits in *Tsc1* $^{+/-}$ mice in the absence of cerebral lesions and seizures. *Annals of Neurology*, *62*(6), 648–655. https://doi.org/10.1002/ana.21317

Gordon, I., Voos, A. C., Bennett, R. H., Bolling, D. Z., Pelphrey, K. A., & Kaiser, M. D. (2013). Brain mechanisms for processing affective touch. *Human Brain Mapping*, *34*(4), 914–922. https://doi.org/10.1002/hbm.21480

Gould, T. D., & Gottesman, I. I. (2006). Psychiatric endophenotypes and the development of valid animal models. *Genes, Brain and Behavior*, *5*(2), 113–119. https://doi.org/10.1111/j.1601-183X.2005.00186.x

Grafman, J., Schwab, K., Warden, D., Pridgen, A., Brown, H. R., & Salazar, A. M. (1996). Frontal lobe injuries, violence, and aggression: a report of the Vietnam Head Injury Study. *Neurology*, *46*(5), 1231–8. https://doi.org/10.1212/WNL.46.5.1231

Grant, R. A., Mitchinson, B., & Prescott, T. J. (2012). The development of whisker control in rats in relation to locomotion. *Developmental Psychobiology*, *54*(2), 151–168. https://doi.org/10.1002/dev.20591

Gray, C. M., & McCormick, D. A. (1996). Chattering cells: superficial pyramidal neurons contributing to the generation of synchronous oscillations in the visual cortex. *Science (New York, N.Y.)*, *274*(5284), 109–13.

Grayton, H. M., Fernandes, C., Rujescu, D., & Collier, D. A. (2012). Copy number variations in neurodevelopmental disorders. *Progress in Neurobiology*, *99*(1), 81–91. https://doi.org/10.1016/J.PNEUROBIO.2012.07.005

Grayton, H. M., Missler, M., Collier, D. A., & Fernandes, C. (2013). Altered Social Behaviours in Neurexin 1α Knockout Mice Resemble Core Symptoms in Neurodevelopmental Disorders. *PLoS ONE*, *8*(6), e67114. https://doi.org/10.1371/journal.pone.0067114

Green, H., McGinnity, Á., Meltzer, H., Ford, T., & Goodman, R. (2005). Mental health of children and young people in Great Britain, 2004.

Green, M. F. (1996). What are the functional consequences of neurocognitive deficits in schizophrenia? *American Journal of Psychiatry*, *153*(3), 321–330. https://doi.org/10.1176/ajp.153.3.321

Green, M. F., & Horan, W. P. (2010). Social Cognition in Schizophrenia. *Current Directions in Psychological Science*, *19*(4), 243–248. https://doi.org/10.1177/0963721410377600

Green, M. F., Kern, R. S., & Heaton, R. K. (2004). Longitudinal studies of cognition and functional outcome in schizophrenia: implications for MATRICS. *Schizophrenia Research*, *72*(1), 41–51. https://doi.org/10.1016/j.schres.2004.09.009

Green, M. F., Penn, D. L., Bentall, R., Carpenter, W. T., Gaebel, W., Gur, R. C., … Heinssen, R. (2008). Social Cognition in Schizophrenia: An NIMH Workshop on Definitions, Assessment, and Research Opportunities. *Schizophrenia Bulletin*, *34*(6), 1211–1220. https://doi.org/10.1093/schbul/sbm145

Green, S. A., Hernandez, L. M., Bowman, H. C., Bookheimer, S. Y., & Dapretto, M. (2017). Sensory over-responsivity and social cognition in ASD: Effects of aversive sensory stimuli and attentional modulation on neural responses to social cues. *Developmental Cognitive Neuroscience*. https://doi.org/10.1016/j.dcn.2017.02.005

Greenough, W. T., Klintsova, A. Y., Irwin, S. A., Galvez, R., Bates, K. E., & Weiler, I. J. (2001). Synaptic regulation of protein synthesis and the fragile X protein. *Proceedings of the National Academy of Sciences of the United States of America*, *98*(13), 7101–6. https://doi.org/10.1073/pnas.141145998

Grosse Wiesmann, C., Schreiber, J., Singer, T., Steinbeis, N., & Friederici, A. D. (2017). White matter maturation is associated with the emergence of Theory of Mind in early childhood. *Nature Communications*, *8*, 14692. https://doi.org/10.1038/ncomms14692

Guić-Robles, E., Valdivieso, C., & Guajardo, G. (1989). Rats can learn a roughness discrimination using only their vibrissal system. *Behavioural Brain Research*, *31*(3), 285–289. https://doi.org/10.1016/0166-4328(89)90011-9

Gusella, J. L., Muir, D., & Tronick, E. Z. (1988). The Effect of Manipulating Maternal Behavior during an Interaction on Three- and Six-Month-Olds' Affect and Attention. *Child Development*, *59*(4), 1111. https://doi.org/10.2307/1130278

Haas, B. W., Barnea-Goraly, N., Sheau, K. E., Yamagata, B., Ullas, S., & Reiss, A. L. (2014). Altered Microstructure Within Social-Cognitive Brain Networks During Childhood in Williams Syndrome. *Cerebral Cortex*, *24*(10), 2796–2806. https://doi.org/10.1093/cercor/bht135

Hadland, K. ., Rushworth, M. F. ., Gaffan, D., & Passingham, R. . (2003). The effect of cingulate lesions on social behaviour and emotion. *Neuropsychologia*, *41*(8), 919–931. https://doi.org/10.1016/S0028-3932(02)00325-1

Hall, C. S., & Whiteman, P. H. (1951). The effects of infantile stimulation upon later emotional stability in the mouse. *Journal of Comparative and Physiological Psychology*, *44*(1), 61–66. https://doi.org/10.1037/h0061899

Hallmayer, J., Cleveland, S., Torres, A., Phillips, J., Cohen, B., Torigoe, T., … Risch, N. (2011). Genetic Heritability and Shared Environmental Factors Among Twin Pairs With Autism. *Archives of General Psychiatry*, *68*(11), 1095. https://doi.org/10.1001/archgenpsychiatry.2011.76

Happé, F., Cook, J. L., & Bird, G. (2017). The Structure of Social Cognition: In(ter)dependence of Sociocognitive Processes. *Annual Review of Psychology*, *68*(1), 243–267. https://doi.org/10.1146/annurev-psych-010416-044046

Happé, F., & Frith, U. (2014). Annual Research Review: Towards a developmental neuroscience of atypical social cognition. *Journal of Child Psychology and Psychiatry*, *55*(6), 553–577. https://doi.org/10.1111/jcpp.12162

Happé, F. G. E. (1994). An advanced test of theory of mind: Understanding of story characters' thoughts and feelings by able autistic, mentally handicapped, and normal children and adults. *Journal of Autism and Developmental Disorders*, *24*(2), 129–154. https://doi.org/10.1007/BF02172093

Hardan, A. Y., Girgis, R. R., Lacerda, A. L. T., Yorbik, O., Kilpatrick, M., Keshavan, M. S., & Minshew, N. J. (2006). Magnetic Resonance Imaging Study of the Orbitofrontal Cortex in Autism. *Journal of Child Neurology*, *21*(10), 866–871. https://doi.org/10.1177/08830738060210100701

Hare, T. A., O'Doherty, J., Camerer, C. F., Schultz, W., & Rangel, A. (2008). Dissociating the role of the orbitofrontal cortex and the striatum in the computation of goal values and prediction errors. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *28*(22), 5623–30. https://doi.org/10.1523/JNEUROSCI.1309-08.2008

Hariri, A. R., Tessitore, A., Mattay, V. S., Fera, F., & Weinberger, D. R. (2002). The amygdala response to emotional stimuli: a comparison of faces and scenes. *Neuroimage*, 17(1), 317-323.

Harrington, A. J., Raissi, A., Rajkovich, K., Berto, S., Kumar, J., Molinaro, G., … Cowan, C. W. (2016). MEF2C regulates cortical inhibitory and excitatory synapses and behaviors

relevant to neurodevelopmental disorders. *eLife*, *5*, e20059. https://doi.org/10.7554/eLife.20059

Harrison, P. J., & Eastwood, S. L. (1998). Preferential involvement of excitatory neurons in medial temporal lobe in schizophrenia. *The Lancet*, *352*(9141), 1669–1673. https://doi.org/10.1016/S0140-6736(98)03341-8

Hasselmo, M. E., Rolls, E. T., & Baylis, G. C. (1989). The role of expression and identity in the face-selective responses of neurons in the temporal visual cortex of the monkey. *Behavioural Brain Research*, *32*(3), 203–218. https://doi.org/10.1016/S0166-4328(89)80054-3

Hata, Y., Butz, S., & Südhof, T. C. (1996). CASK: a novel dlg/PSD95 homolog with an N-terminal calmodulin-dependent protein kinase domain identified by interaction with neurexins. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *16*(8), 2488–94.

Hata, Y., Davletov, B., Petrenko, A. G., Jahn, R., & Südhof, T. C. (1993). Interaction of synaptotagmin with the cytoplasmic domains of neurexins. *Neuron*, *10*(2), 307–315. https://doi.org/10.1016/0896-6273(93)90320-Q

Hayden, B. Y., Parikh, P. C., Deaner, R. O., & Platt, M. L. (2007). Economic principles motivating social attention in humans. *Proceedings. Biological Sciences*, *274*(1619), 1751–6. https://doi.org/10.1098/rspb.2007.0368

Hayes, L. A., & Watson, J. S. (1981). Neonatal imitation: Fact or artifact? *Developmental Psychology*, *17*(5), 655–660. https://doi.org/10.1037/0012-1649.17.5.655

Helmbold, K., Zvyagintsev, M., Dahmen, B., Bubenzer-Busch, S., Gaber, T. J., Crockett, M.

J., … Zepf, F. D. (2015). Effects of serotonin depletion on punishment processing in the orbitofrontal and anterior cingulate cortices of healthy women. *European Neuropsychopharmacology*, *25*(6), 846–856. https://doi.org/10.1016/J.EURONEURO.2015.02.007

Helmeke, C., Seidel, K., Poeggel, G., Bredy, T. W., Abraham, A., & Braun, K. (2009). Paternal deprivation during infancy results in dendrite- and time-specific changes of dendritic development and spine formation in the orbitofrontal cortex of the biparental rodent Octodon degus. *Neuroscience*, *163*(3), 790–798. https://doi.org/10.1016/j.neuroscience.2009.07.008

Henry, J. D., von Hippel, W., Molenberghs, P., Lee, T., & Sachdev, P. S. (2015). Clinical assessment of social cognitive function in neurological disorders. *Nature Reviews Neurology*, *12*(1), 28–39. https://doi.org/10.1038/nrneurol.2015.229

Hensch, T. K. (2005). Critical period plasticity in local cortical circuits. *Nature Reviews Neuroscience*, *6*(11), 877–888. https://doi.org/10.1038/nrn1787

Herbert, M. R., Ziegler, D. A., Makris, N., Filipek, P. A., Kemper, T. L., Normandin, J. J., … Caviness, V. S. (2004). Localization of white matter volume increase in autism and developmental language disorder. *Annals of Neurology*, *55*(4), 530–540. https://doi.org/10.1002/ana.20032

Hermes, G., Li, N., Duman, C., & Duman, R. (2011). Post-weaning chronic social isolation produces profound behavioral dysregulation with decreases in prefrontal cortex synaptic-associated protein expression in female rats. *Physiology & Behavior*, *104*(2), 354–359. https://doi.org/10.1016/J.PHYSBEH.2010.12.019

Heyes, C. (2012). What's social about social learning? *Journal of Comparative Psychology,*

126(2), 193–202. https://doi.org/10.1037/a0025180

Heyes, C. M. (1993). Anecdotes, training, trapping and triangulating: do animals attribute mental states? *Animal Behaviour*, *46*(1), 177–188. https://doi.org/10.1006/ANBE.1993.1173

Hill, E. L. (2004). Executive dysfunction in autism. *Trends in Cognitive Sciences*, *8*(1), 26–32. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/14697400

Hilton, C., Graver, K., & LaVesser, P. (2007). Relationship between social competence and sensory processing in children with high functioning autism spectrum disorders. *Research in Autism Spectrum Disorders*, *1*(2), 164–173. https://doi.org/10.1016/J.RASD.2006.10.002

Hilton, C. L., Harper, J. D., Kueker, R. H., Lang, A. R., Abbacchi, A. M., Todorov, A., & LaVesser, P. D. (2010). Sensory Responsiveness as a Predictor of Social Severity in Children with High Functioning Autism Spectrum Disorders. *Journal of Autism and Developmental Disorders*, *40*(8), 937–945. https://doi.org/10.1007/s10803-010-0944-8

Hol, T., Van den Berg, C. L., Van Ree, J. M., & Spruijt, B. M. (1999). Isolation during the play period in infancy decreases adult social interactions in rats. *Behavioural Brain Research*, *100*(1–2), 91–97. https://doi.org/10.1016/S0166-4328(98)00116-8

Holloway, K. S., & Suter, R. B. (2004). Play deprivation without social isolation: Housing controls. *Developmental Psychobiology*, *44*(1), 58–67. https://doi.org/10.1002/dev.10151

Holt-Lunstad, J., Smith, T. B., & Layton, J. B. (2010). Social Relationships and Mortality Risk: A Meta-analytic Review. *PLoS Medicine*, *7*(7), e1000316.

https://doi.org/10.1371/journal.pmed.1000316

Holtmaat, A., & Svoboda, K. (2009). Experience-dependent structural synaptic plasticity in the mammalian brain. *Nature Reviews Neuroscience*, *10*(9), 647–658. https://doi.org/10.1038/nrn2699

Holy, T. E., & Guo, Z. (2005). Ultrasonic songs of male mice. *PLoS Biology*, *3*(12), e386. https://doi.org/10.1371/journal.pbio.0030386

Hoptman, M. J., D'Angelo, D., Catalano, D., Mauro, C. J., Shehzad, Z. E., Kelly, A. M. C., … Milham, M. P. (2010). Amygdalofrontal Functional Disconnectivity and Aggression in Schizophrenia. *Schizophrenia Bulletin*, *36*(5), 1020–1028. https://doi.org/10.1093/schbul/sbp012

Hori, M., Yamada, K., Ohnishi, J., Sakamoto, S., Furuie, H., Murakami, K., & Ichitani, Y. (2014). Tickling during adolescence alters fear-related and cognitive behaviors in rats after prolonged isolation. *Physiology & Behavior*, *131*, 62–67. https://doi.org/10.1016/j.physbeh.2014.04.008

Hubel, D. H., & Wiesel, T. N. (1964). Effects of monocular deprivation in kittens. *Naunyn-Schmiedebergs Archiv Fur Experimentelle Pathologie Und Pharmakologie*, *248*(6), 492–497. https://doi.org/10.1007/BF00348878

Hughes, C. (1998). Executive function in preschoolers: Links with theory of mind and verbal ability. *British Journal of Developmental Psychology*, *16*(2), 233–253. https://doi.org/10.1111/j.2044-835X.1998.tb00921.x

Hughes, C., & Ensor, R. (2007). Executive function and theory of mind: Predictive relations from ages 2 to 4. *Developmental Psychology*, *43*(6), 1447–1459.

https://doi.org/10.1037/0012-1649.43.6.1447

Hughes, C., Russell, J., & Robbins, T. W. (1994). Evidence for executive dysfunction in autism. *Neuropsychologia*, *32*(4), 477–92.

Humphreys, A. P., & Einon, D. F. (1981). Play as a reinforcer for maze-learning in juvenile rats. *Animal Behaviour*, *29*(1), 259–270. https://doi.org/10.1016/S0003-3472(81)80173-X

Hurst, J. L., Payne, C. E., Nevison, C. M., Marie, A. D., Humphries, R. E., Robertson, D. H. L., … Beynon, R. J. (2001). Individual recognition in mice mediated by major urinary proteins. *Nature*, *414*(6864), 631–634. https://doi.org/10.1038/414631a

Hutsler, J. J., & Zhang, H. (2010). Increased dendritic spine densities on cortical projection neurons in autism spectrum disorders. *Brain Research*, *1309*, 83–94. https://doi.org/10.1016/j.brainres.2009.09.120

Hutson, K. A., & Masterton, R. B. (1986). The sensory contribution of a single vibrissa's cortical barrel. *Journal of Neurophysiology*, *56*(4), 1196–223. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/3783236

Huttenlocher, P. R. (1979). Synaptic density in human frontal cortex — Developmental changes and effects of aging. *Brain Research*, *163*(2), 195–205. https://doi.org/10.1016/0006-8993(79)90349-4

Huttenlocher, P. R. (1990). Morphometric study of human cerebral cortex development. *Neuropsychologia*, *28*(6), 517–527. https://doi.org/10.1016/0028-3932(90)90031-I

Huttenlocher, P. R., de Courten, C., Garey, L. J., & Van der Loos, H. (1982). Synaptogenesis in human visual cortex — evidence for synapse elimination during normal development.

Neuroscience Letters, *33*(3), 247–252. https://doi.org/10.1016/0304-3940(82)90379-2

Hynes, C. A., Baird, A. A., & Grafton, S. T. (2006). Differential role of the orbital frontal lobe in emotional versus cognitive perspective-taking. *Neuropsychologia*, *44*(3), 374–383. https://doi.org/10.1016/J.NEUROPSYCHOLOGIA.2005.06.011

Insel, T. R., & Cuthbert, B. N. (2015). Medicine. Brain disorders? Precisely. *Science (New York, N.Y.)*, *348*(6234), 499–500. https://doi.org/10.1126/science.aab2358

Insel, T. R., & Hulihan, T. J. (1995). A gender-specific mechanism for pair bonding: Oxytocin and partner preference formation in monogamous voles. *Behavioral Neuroscience*, *109*(4), 782–789. https://doi.org/10.1037/0735-7044.109.4.782

Irwin, S. A., Patel, B., Idupulapati, M., Harris, J. B., Crisostomo, R. A., Larsen, B. P., … Greenough, W. T. (2001). Abnormal dendritic spine characteristics in the temporal and visual cortices of patients with fragile-X syndrome: A quantitative examination. *American Journal of Medical Genetics*, *98*(2), 161–167. https://doi.org/10.1002/1096-8628(20010115)98:2<161::AID-AJMG1025>3.0.CO;2-B

Israel, S., Lerer, E., Shalev, I., Uzefovsky, F., Riebold, M., Laiba, E., … Ebstein, R. P. (2009). The Oxytocin Receptor (OXTR) Contributes to Prosocial Fund Allocations in the Dictator Game and the Social Value Orientations Task. *PLoS ONE*, *4*(5), e5535. https://doi.org/10.1371/journal.pone.0005535

Itahashi, T., Yamada, T., Nakamura, M., Watanabe, H., Yamagata, B., Jimbo, D., … Hashimoto, R. (2015). Linked alterations in gray and white matter morphology in adults with high-functioning autism spectrum disorder: A multimodal brain imaging study. *NeuroImage: Clinical*, *7*, 155–169. https://doi.org/10.1016/j.nicl.2014.11.019

Itskov, V., Hansel, D., & Tsodyks, M. (2011). Short-Term Facilitation may Stabilize Parametric Working Memory Trace. *Frontiers in Computational Neuroscience*, *5*, 40. https://doi.org/10.3389/fncom.2011.00040

Izquierdo, A., & Murray, E. A. (2005). Opposing effects of amygdala and orbital prefrontal cortex lesions on the extinction of instrumental responding in macaque monkeys. *European Journal of Neuroscience*, *22*(9), 2341–2346. https://doi.org/10.1111/j.1460-9568.2005.04434.x

Jackman, S. L., & Regehr, W. G. (2017). The Mechanisms and Functions of Synaptic Facilitation. *Neuron*, *94*(3), 447–464. https://doi.org/10.1016/J.NEURON.2017.02.047

Jackman, S. L., Turecek, J., Belinsky, J. E., & Regehr, W. G. (2016). The calcium sensor synaptotagmin 7 is required for synaptic facilitation. *Nature*, *529*(7584), 88–91. https://doi.org/10.1038/nature16507

Jackson, M. B., Konnerth, A., & Augustine, G. J. (1991). Action potential broadening and frequency-dependent facilitation of calcium signals in pituitary nerve terminals. *Proceedings of the National Academy of Sciences of the United States of America*, *88*(2), 380–4.

Jackson, S. A. W., Horst, N. K., Pears, A., Robbins, T. W., & Roberts, A. C. (2016). Role of the Perigenual Anterior Cingulate and Orbitofrontal Cortex in Contingency Learning in the Marmoset. *Cerebral Cortex*, *26*(7), 3273–3284. https://doi.org/10.1093/cercor/bhw067

Jacquemont, M.-L., Sanlaville, D., Redon, R., Raoul, O., Cormier-Daire, V., Lyonnet, S., … Philippe, A. (2006). Array-based comparative genomic hybridisation identifies high frequency of cryptic chromosomal rearrangements in patients with syndromic autism

spectrum disorders. *Journal of Medical Genetics*, *43*(11), 843–9. https://doi.org/10.1136/jmg.2006.043166

Jalbrzikowski, M., Villalon-Reina, J. E., Karlsgodt, K. H., Senturk, D., Chow, C., Thompson, P. M., & Bearden, C. E. (2014). Altered white matter microstructure is associated with social cognition and psychotic symptoms in 22q11.2 microdeletion syndrome. *Frontiers in Behavioral Neuroscience*, *8*, 393. https://doi.org/10.3389/fnbeh.2014.00393

Jamain, S., Betancur, C., Quach, H., Philippe, A., Fellous, M., Giros, B., … Paris Autism Research International Sibpair (PARIS) Study, the P. A. R. I. S. (PARIS). (2002). Linkage and association of the glutamate receptor 6 gene with autism. *Molecular Psychiatry*, *7*(3), 302–10. https://doi.org/10.1038/sj.mp.4000979

Jamain, S., Quach, H., Betancur, C., Råstam, M., Colineaux, C., Gillberg, I. C., … Maldergem, L. Van. (2003). Mutations of the X-linked genes encoding neuroligins NLGN3 and NLGN4 are associated with autism. *Nature Genetics*, *34*(1), 27–29. https://doi.org/10.1038/ng1136

Jamain, S., Radyushkin, K., Hammerschmidt, K., Granon, S., Boretius, S., Varoqueaux, F., … Brose, N. (2008). Reduced social interaction and ultrasonic communication in a mouse model of monogenic heritable autism. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(5), 1710–5. https://doi.org/10.1073/pnas.0711555105

Jang, W. H., Choi, S. H., Jeong, J. Y., Park, J.-H., Kim, S.-J., & Seog, D.-H. (2014). Neuronal cell-surface protein neurexin 1 interaction with multi-PDZ domain protein MUPP1. *Bioscience, Biotechnology, and Biochemistry*, *78*(4), 644–646. https://doi.org/10.1080/09168451.2014.890031

Jarrold, C. (2003). A Review of Research into Pretend Play in Autism. *Autism*, *7*(4), 379–390. https://doi.org/10.1177/1362361303007004004

Jenkins, L. M., Andrewes, D. G., Nicholas, C. L., Drummond, K. J., Moffat, B. A., Phal, P., … Kessels, R. P. C. (2014). Social cognition in patients following surgery to the prefrontal cortex. *Psychiatry Research: Neuroimaging*, *224*(3), 192–203. https://doi.org/10.1016/j.pscychresns.2014.08.007

Jentsch, J. D., Trantham-Davidson, H., Jairl, C., Tinsley, M., Cannon, T. D., & Lavin, A. (2009). Dysbindin modulates prefrontal cortical glutamatergic circuits and working memory function in mice. *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology*, *34*(12), 2601–8. https://doi.org/10.1038/npp.2009.90

Jeon, D., & Shin, H.-S. (2011). A Mouse Model for Observational Fear Learning and the Empathetic Response. In *Current Protocols in Neuroscience* (Vol. Chapter 8, p. Unit 8.27). Hoboken, NJ, USA: John Wiley & Sons, Inc. https://doi.org/10.1002/0471142301.ns0827s57

Jiang-Xie, L.-F., Liao, H.-M., Chen, C.-H., Chen, Y.-T., Ho, S.-Y., Lu, D.-H., … Gau, S. S.-F. (2014a). Autism-associated gene Dlgap2 mutant mice demonstrate exacerbated aggressive behaviors and orbitofrontal cortex deficits. *Molecular Autism*, *5*(1), 32. https://doi.org/10.1186/2040-2392-5-32

Jiang, Y., & Ehlers, M. D. (2013a). Modeling Autism by SHANK Gene Mutations in Mice. *Neuron*, *78*(1), 8–27. https://doi.org/10.1016/j.neuron.2013.03.016

Johnson, M. H., Dziurawiec, S., Ellis, H., & Morton, J. (1991). Newborns' preferential tracking of face-like stimuli and its subsequent decline. *Cognition*, *40*(1–2), 1–19.

https://doi.org/10.1016/0010-0277(91)90045-6

Johnson, M. H., Grossmann, T., & Kadosh, K. C. (2009). Mapping functional brain development: Building a social brain through interactive specialization. *Developmental Psychology*, *45*(1), 151–159. https://doi.org/10.1037/a0014548

Jones, S. S. (2009). The development of imitation in infancy. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *364*(1528), 2325–35. https://doi.org/10.1098/rstb.2009.0045

Jones, W., Bellugi, U., Lai, Z., Chiles, M., Reilly, J., Lincoln, A., & Adolphs, R. (2000). II. Hypersociability in Williams Syndrome. *Journal of Cognitive Neuroscience*, *12*(supplement 1), 30–46. https://doi.org/10.1162/089892900561968

Joseph, R. M., & Tager–Flusberg, H. (2004). The relationship of theory of mind and executive functions to symptom type and severity in children with autism. *Development and Psychopathology*, *16*(1), 137–155. https://doi.org/10.1017/S095457940404444X

Just, M. A., Cherkassky, V. L., Keller, T. A., & Minshew, N. J. (2004). Cortical activation and synchronization during sentence comprehension in high-functioning autism: evidence of underconnectivity. *Brain*, *127*(8), 1811–1821. https://doi.org/10.1093/brain/awh199

Kaiser, M. D., Yang, D. Y.-J., Voos, A. C., Bennett, R. H., Gordon, I., Pretzsch, C., … Pelphrey, K. A. (2016). Brain Mechanisms for Processing Affective (and Nonaffective) Touch Are Atypical in Autism. *Cerebral Cortex (New York, N.Y. : 1991)*, *26*(6), 2705–14. https://doi.org/10.1093/cercor/bhv125

Kamiya, H., & Zucker, R. S. (1994). Residual Ca2 + and short-term synaptic plasticity.

Nature, *371*(6498), 603–606. https://doi.org/10.1038/371603a0

Kana, R. K., Keller, T. A., Minshew, N. J., & Just, M. A. (2007). Inhibitory Control in High-Functioning Autism: Decreased Activation and Underconnectivity in Inhibition Networks. *Biological Psychiatry*, *62*(3), 198–206. https://doi.org/10.1016/J.BIOPSYCH.2006.08.004

Kanwisher, N., McDermott, J., & Chun, M. M. (1997). The Fusiform Face Area: A Module in Human Extrastriate Cortex Specialized for Face Perception. *Journal of Neuroscience*, *17*(11).

Kanwisher, N., McDermott, J., Chun, M. M., Bramati, I. E., Mourão-Miranda, J., Andreiuolo, P. A., & Pessoa, L. (1997). The fusiform face area: a module in human extrastriate cortex specialized for face perception. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *17*(11), 4302–11.

Karmiloff-Smith, A. (1998). Development itself is the key to understanding developmental disorders. *Trends in Cognitive Sciences*, *2*(10), 389–398. https://doi.org/10.1016/S1364-6613(98)01230-3

Karmiloff-Smith, A. (2009). Nativism versus neuroconstructivism: Rethinking the study of developmental disorders. *Developmental Psychology*, *45*(1), 56–63. https://doi.org/10.1037/a0014506

Karmiloff-Smith, A., D'Souza, D., Dekker, T. M., Van Herwegen, J., Xu, F., Rodic, M., & Ansari, D. (2012). Genetic and environmental vulnerabilities in children with neurodevelopmental disorders. *Proceedings of the National Academy of Sciences of the United States of America*, *109 Suppl 2*(Supplement 2), 17261–5. https://doi.org/10.1073/pnas.1121087109

Karmiloff-Smith, A., Scerif, G., & Ansari, D. (2003). Double dissociations in developmental disorders? Theoretically misconceived, empirically dubious. *Cortex; a Journal Devoted to the Study of the Nervous System and Behavior*, *39*(1), 161–3.

Katayama, N., Yamamori, S., Fukaya, M., Kobayashi, S., Watanabe, M., Takahashi, M., & Manabe, T. (2017). SNAP-25 phosphorylation at Ser187 regulates synaptic facilitation and short-term plasticity in an age-dependent manner. *Scientific Reports*, *7*(1), 7996. https://doi.org/10.1038/s41598-017-08237-x

Katayama, Y., Nishiyama, M., Shoji, H., Ohkawa, Y., Kawamura, A., Sato, T., … Nakayama, K. I. (2016). CHD8 haploinsufficiency results in autistic-like phenotypes in mice. *Nature*, *537*(7622), 675–679. https://doi.org/10.1038/nature19357

Kattenstroth, G., Tantalaki, E., Südhof, T. C., Gottmann, K., & Missler, M. (2004). Postsynaptic N-methyl-D-aspartate receptor function requires α-neurexins. Proceedings of the National Academy of Sciences of the United States of America, 101(8), 2607-2612.

Katz, B., & Miledi, R. (1968). The role of calcium in neuromuscular facilitation. *The Journal of Physiology*, *195*(2), 481–492. https://doi.org/10.1113/jphysiol.1968.sp008469

Kavalali, E. T. (2014). The mechanisms and functions of spontaneous neurotransmitter release. *Nature Reviews Neuroscience*, *16*(1), 5–16. https://doi.org/10.1038/nrn3875

Ke, X., Tang, T., Hong, S., Hang, Y., Zou, B., Li, H., … Liu, Y. (2009). White matter impairments in autism, evidence from voxel-based morphometry and diffusion tensor imaging. *Brain Research*, *1265*, 171–177. https://doi.org/10.1016/J.BRAINRES.2009.02.013

Kenny, E. M., Cormican, P., Furlong, S., Heron, E., Kenny, G., Fahey, C., … Morris, D. W. (2014). Excess of rare novel loss-of-function variants in synaptic genes in schizophrenia and autism spectrum disorders. *Molecular Psychiatry*, *19*(8), 872–879. https://doi.org/10.1038/mp.2013.127

Kern, J. K., Trivedi, M. H., Garver, C. R., Grannemann, B. D., Andrews, A. A., Savla, J. S., … Schroeder, J. L. (2006). The pattern of sensory processing abnormalities in autism. *Autism*, *10*(5), 480–494. https://doi.org/10.1177/1362361306066564

Kern, J. K., Trivedi, M. H., Grannemann, B. D., Garver, C. R., Johnson, D. G., Andrews, A. A., … Schroeder, J. L. (2007). Sensory correlations in autism. *Autism*, *11*(2), 123–134. https://doi.org/10.1177/1362361307075702

Khan, S., Gramfort, A., Shetty, N. R., Kitzbichler, M. G., Ganesan, S., Moran, J. M., … Kenet, T. (2013). Local and long-range functional connectivity is reduced in concert in autism spectrum disorders. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(8), 3107–12. https://doi.org/10.1073/pnas.1214533110

Kida, T., & Shinohara, K. (2013). Gentle touch activates the anterior prefrontal cortex: An NIRS study. *Neuroscience Research*, *76*(1–2), 76–82. https://doi.org/10.1016/j.neures.2013.03.006

Kientz, M. A., & Dunn, W. (1997). A Comparison of the Performance of Children With and Without Autism on the Sensory Profile. *American Journal of Occupational Therapy*, *51*(7), 530–537. https://doi.org/10.5014/ajot.51.7.530

Kim, H.-G., Kishikawa, S., Higgins, A. W., Seong, I.-S., Donovan, D. J., Shen, Y., … Gusella, J. F. (2008). Disruption of Neurexin 1 Associated with Autism Spectrum Disorder. *The American Journal of Human Genetics*, *82*(1), 199–207.

https://doi.org/10.1016/J.AJHG.2007.09.011

Kirov, G., Rujescu, D., Ingason, A., Collier, D. A., O'Donovan, M. C., & Owen, M. J. (2009). Neurexin 1 (NRXN1) deletions in schizophrenia. *Schizophrenia Bulletin*, *35*(5), 851–4. https://doi.org/10.1093/schbul/sbp079

Klaassen, T., Riedel, W. J., Deutz, N. E. P., & Van Praag, H. M. (2002). Mood congruent memory bias induced by tryptophan depletion. *Psychological Medicine*, *32*(1), 167–172. https://doi.org/10.1017/S003329170100438X

Klavir, O., Prigge, M., Sarel, A., Paz, R., & Yizhar, O. (2017). Manipulating fear associations via optogenetic modulation of amygdala inputs to prefrontal cortex. *Nature Neuroscience*. https://doi.org/10.1038/nn.4523

Klingauf, J., & Neher, E. (1997). Modeling Buffered Ca2+ Diffusion Near the Membrane: Implications for Secretion in Neuroendocrine Cells. *Biophysical Journal*, *72*(2), 674–690. https://doi.org/10.1016/S0006-3495(97)78704-6

Knafo, A., Israel, S., Darvasi, A., Bachner-Melman, R., Uzefovsky, F., Cohen, L., … Ebstein, R. P. (2008). Individual differences in allocation of funds in the dictator game associated with length of the arginine vasopressin 1a receptor RS3 promoter region and correlation between RS3 length and hippocampal mRNA. *Genes, Brain and Behavior*, *7*(3), 266–275. https://doi.org/10.1111/j.1601-183X.2007.00341.x

Knafo, A., & Plomin, R. (2006). Prosocial behavior from early to middle childhood: Genetic and environmental influences on stability and change. *Developmental Psychology*, *42*(5), 771–786. https://doi.org/10.1037/0012-1649.42.5.771

Knafo, A., Zahn-Waxler, C., Hulle, C. Van, Robinson, J. L., & Rhee, S. H. (n.d.). The

Developmental Origins of a Disposition Toward Empathy: Genetic and Environmental Contributions. https://doi.org/10.1037/a0014179

Knudsen, E. I. (2004). Sensitive Periods in the Development of the Brain and Behavior. *Journal of Cognitive Neuroscience*, *16*(8), 1412–1425. https://doi.org/10.1162/0898929042304796

Knudsen, E. I., Knudsen, P. F., & Esterly, S. D. (1984). A critical period for the recovery of sound localization accuracy following monaural occlusion in the barn owl. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *4*(4), 1012–20.

Knutson, B., Burgdorf, J., & Panksepp, J. (1998a). Anticipation of play elicits high-frequency ultrasonic vocalizations in young rats. *Journal of Comparative Psychology*, *112*(1), 65–73. https://doi.org/10.1037/0735-7036.112.1.65

Knutson, B., Burgdorf, J., & Panksepp, J. (1999). High-Frequency Ultrasonic Vocalizations Index Conditioned Pharmacological Reward in Rats. *Physiology & Behavior*, *66*(4), 639–643. https://doi.org/10.1016/S0031-9384(98)00337-0

Knutson, B., Burgdorf, J., & Panksepp, J. (2002). Ultrasonic vocalizations as indices of affective states in rats. *Psychological Bulletin*, *128*(6), 961–977. https://doi.org/10.1037/0033-2909.128.6.961

Ko, J., Fuccillo, M. V., Malenka, R. C., & Südhof, T. C. (2009). LRRTM2 Functions as a Neurexin Ligand in Promoting Excitatory Synapse Formation. *Neuron*, *64*(6), 791–798. https://doi.org/10.1016/J.NEURON.2009.12.012

Kochanska, G., Gross, J. N., Lin, M.-H., & Nichols, K. E. (2002). Guilt in Young Children: Development, Determinants, and Relations with a Broader System of Standards. *Child*

Development, *73*(2), 461–482. https://doi.org/10.1111/1467-8624.00418

Koenig, J. H., & Ikeda, K. (1999). Contribution of active zone subpopulation of vesicles to evoked and spontaneous release. *Journal of Neurophysiology*, *81*(4), 1495–505.

Koike, H., Arguello, P. A., Kvajo, M., Karayiorgou, M., & Gogos, J. A. (2006). Disc1 is mutated in the 129S6/SvEv strain and modulates working memory in mice. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(10), 3693–7. https://doi.org/10.1073/pnas.0511189103

Kolluri, N., Sun, Z., Sampson, A. R., & Lewis, D. A. (2005). Lamina-Specific Reductions in Dendritic Spine Density in the Prefrontal Cortex of Subjects With Schizophrenia. *American Journal of Psychiatry*, *162*(6), 1200–1202. https://doi.org/10.1176/appi.ajp.162.6.1200

Kondo, H., Saleem, K. S., & Price, J. L. (2003). Differential connections of the temporal pole with the orbital and medial prefrontal networks in macaque monkeys. *The Journal of Comparative Neurology*, *465*(4), 499–523. https://doi.org/10.1002/cne.10842

Kondo, H., & Witter, M. P. (2014). Topographic organization of orbitofrontal projections to the parahippocampal region in rats. *Journal of Comparative Neurology*, *522*(4), 772–793. https://doi.org/10.1002/cne.23442

Koolhaas, J. M., Coppens, C. M., de Boer, S. F., Buwalda, B., Meerlo, P., & Timmermans, P. J. A. (2013). The resident-intruder paradigm: a standardized test for aggression, violence and social stress. *Journal of Visualized Experiments : JoVE*, (77), e4367. https://doi.org/10.3791/4367

Korkmaz, B. (2011). Theory of Mind and Neurodevelopmental Disorders of Childhood.

Pediatric Research 2011 69:5, Part 2 of 2.

Korn, H., & Faber, D. S. (1998). Quantal analysis and long-term potentiation. *Comptes Rendus de l'Academie Des Sciences. Serie III, Sciences de La Vie*, *321*(2–3), 125–30.

Kosfeld, M., Heinrichs, M., Zak, P. J., Fischbacher, U., & Fehr, E. (2005). Oxytocin increases trust in humans. *Nature*, *435*(7042), 673–676. https://doi.org/10.1038/nature03701

Koshino, H., Carpenter, P. A., Minshew, N. J., Cherkassky, V. L., Keller, T. A., & Just, M. A. (2005). Functional connectivity in an fMRI working memory task in high-functioning autism. *NeuroImage*, *24*(3), 810–821. https://doi.org/10.1016/J.NEUROIMAGE.2004.09.028

Krettek, J. E., & Price, J. L. (1977). The cortical projections of the mediodorsal nucleus and adjacent thalamic nuclei in the rat. *The Journal of Comparative Neurology*, *171*(2), 157–191. https://doi.org/10.1002/cne.901710204

Kringelbach, M. L., O'Doherty, J., Rolls, E. T., & Andrews, C. (2003). Activation of the Human Orbitofrontal Cortex to a Liquid Food Stimulus is Correlated with its Subjective Pleasantness. *Cerebral Cortex*, *13*(10), 1064–1071. https://doi.org/10.1093/cercor/13.10.1064

Kuhaneck, H. M., & Britner, P. A. (2013). A Preliminary Investigation of the Relationship between Sensory Processing and Social Play in Autism Spectrum Disorder. *OTJR: Occupation, Participation and Health*, *33*(3), 159–167. https://doi.org/10.3928/15394492-20130614-04

Kuhlman, S. J., Olivas, N. D., Tring, E., Ikrar, T., Xu, X., & Trachtenberg, J. T. (2013). A

disinhibitory microcircuit initiates critical-period plasticity in the visual cortex. *Nature*, *501*(7468), 543–546. https://doi.org/10.1038/nature12485

Kumaran, D., Banino, A., Blundell, C., Hassabis, D., & Dayan, P. (2016). Computations Underlying Social Hierarchy Learning: Distinct Neural Mechanisms for Updating and Representing Self-Relevant Information. *Neuron*, *92*(5), 1135–1147. https://doi.org/10.1016/j.neuron.2016.10.052

Kumaran, D., Melo, H. L., & Duzel, E. (2012). The emergence and representation of knowledge about social and nonsocial hierarchies. *Neuron*, *76*(3), 653–66. https://doi.org/10.1016/j.neuron.2012.09.035

Kummer, K., Klement, S., Eggart, V., Mayr, M. J., Saria, A., & Zernig, G. (2011). Conditioned place preference for social interaction in rats: contribution of sensory components. *Frontiers in Behavioral Neuroscience*, *5*, 80. https://doi.org/10.3389/fnbeh.2011.00080

Kuramoto, E., Iwai, H., Yamanaka, A., Ohno, S., Seki, H., Tanaka, Y. R., … Goto, T. (2017). Dorsal and ventral parts of thalamic nucleus submedius project to different areas of rat orbitofrontal cortex: a single neuron-tracing study using virus vectors. *Journal of Comparative Neurology*. https://doi.org/10.1002/cne.24306

Kuswanto, C. N., Teh, I., Lee, T.-S., & Sim, K. (2012). Diffusion tensor imaging findings of white matter changes in first episode schizophrenia: a systematic review. *Clinical Psychopharmacology and Neuroscience : The Official Scientific Journal of the Korean College of Neuropsychopharmacology*, *10*(1), 13–24. https://doi.org/10.9758/cpn.2012.10.1.13

Kvajo, M., McKellar, H., Arguello, P. A., Drew, L. J., Moore, H., MacDermott, A. B., …

Gogos, J. A. (2008). A mutation in mouse Disc1 that models a schizophrenia risk allele leads to specific alterations in neuronal architecture and cognition. *Proceedings of the National Academy of Sciences*, *105*(19), 7076–7081. https://doi.org/10.1073/pnas.0802615105

Kvajo, M., McKellar, H., Drew, L. J., Lepagnol-Bestel, A.-M., Xiao, L., Levy, R. J., … Gogos, J. A. (2011a). Altered axonal targeting and short-term plasticity in the hippocampus of Disc1 mutant mice. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(49), E1349-58. https://doi.org/10.1073/pnas.1114113108

Laarakker, M. C., Reinders, N. R., Bruining, H., Ophoff, R. A., & Kas, M. J. H. (2012). Sex-Dependent Novelty Response in Neurexin-1α Mutant Mice. *PLoS ONE*, *7*(2), e31503. https://doi.org/10.1371/journal.pone.0031503

Lacroix, A., Guidetti, M., Rogé, B., & Reilly, J. (2009). Recognition of emotional and nonemotional facial expressions: A comparison between Williams syndrome and autism. *Research in Developmental Disabilities*, *30*(5), 976–985. https://doi.org/10.1016/J.RIDD.2009.02.002

Lambo, M. E., & Turrigiano, G. G. (2013). Synaptic and intrinsic homeostatic mechanisms cooperate to increase L2/3 pyramidal neuron excitability during a late phase of critical period plasticity. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *33*(20), 8810–9. https://doi.org/10.1523/JNEUROSCI.4502-12.2013

Lamm, C., Silani, G., & Singer, T. (2015). Distinct neural networks underlying empathy for pleasant and unpleasant touch. *Cortex*, *70*, 79–89. https://doi.org/10.1016/j.cortex.2015.01.021

Lander, S. S., Linder-Shacham, D., & Gaisler-Salomon, I. (2017). Differential effects of social isolation in adolescent and adult mice on behavior and cortical gene expression. *Behavioural Brain Research*, *316*, 245–254. https://doi.org/10.1016/j.bbr.2016.09.005

Landers, M. S., & Sullivan, R. M. (1999). Vibrissae-evoked behavior and conditioning before functional ontogeny of the somatosensory vibrissae cortex. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *19*(12), 5131–7.

Lass, N. J., Hughes, K. R., Bowyer, M. D., Waters, L. T., & Bourne, V. T. (1976). Speaker sex identification from voiced, whispered, and filtered isolated vowels. *The Journal of the Acoustical Society of America*, *59*(3), 675–678. https://doi.org/10.1121/1.380917

Laumonnier, F., Bonnet-Brilhault, F., Gomot, M., Blanc, R., David, A., Moizard, M.-P., … Briault, S. (2004). X-Linked Mental Retardation and Autism Are Associated with a Mutation in the NLGN4 Gene, a Member of the Neuroligin Family. *The American Journal of Human Genetics*, *74*(3), 552–557. https://doi.org/10.1086/382137

Laws, G., & Bishop, D. V. M. (2004). Pragmatic language impairment and social deficits in Williams syndrome: a comparison with Down's syndrome and specific language impairment. *International Journal of Language & Communication Disorders*, *39*(1), 45–64. https://doi.org/10.1080/13682820310001615797

LeDoux, J. E., Sakaguchi, A. K. I. R. A., & Reis, D. J. (1984). Subcortical efferent projections of the medial geniculate nucleus mediate emotional responses conditioned to acoustic stimuli. *Journal of Neuroscience*, 4(3), 683-698.

Lee, J. E., Bigler, E. D., Alexander, A. L., Lazar, M., DuBray, M. B., Chung, M. K., … Lainhart, J. E. (2007). Diffusion tensor imaging of white matter in the superior temporal gyrus and temporal stem in autism. *Neuroscience Letters*, *424*(2), 127–132.

https://doi.org/10.1016/J.NEULET.2007.07.042

Lee, L.-J., Chen, W.-J., Chuang, Y.-W., & Wang, Y.-C. (2009). Neonatal whisker trimming causes long-lasting changes in structure and function of the somatosensory system. *Experimental Neurology*, *219*(2), 524–532. https://doi.org/10.1016/j.expneurol.2009.07.012

Lee, T. M. C., Ip, A. K. Y., Wang, K., Xi, C., Hu, P., Mak, H. K. F., … Chan, C. C. H. (2010). Faux pas deficits in people with medial frontal lesions as related to impaired understanding of a speaker's mental state. *Neuropsychologia*, *48*(6), 1670–1676. https://doi.org/10.1016/J.NEUROPSYCHOLOGIA.2010.02.012

Leekam, S. R., & Ramsden, C. A. H. (2006). Dyadic Orienting and Joint Attention in Preschool Children with Autism. *Journal of Autism and Developmental Disorders*, *36*(2), 185–197. https://doi.org/10.1007/s10803-005-0054-1

Lejeune, J., Gautier, M., & Turpin, R. (1959). [Study of somatic chromosomes from 9 mongoloid children]. *Comptes Rendus Hebdomadaires Des Seances de l'Academie Des Sciences*, *248*(11), 1721–2.

Lenschow, C., & Brecht, M. (2015). Barrel Cortex Membrane Potential Dynamics in Social Touch. *Neuron*, *85*(4), 718–725. https://doi.org/10.1016/j.neuron.2014.12.059

Leopold, D. A., & Maier, A. (2012). Ongoing physiological processes in the cerebral cortex. *NeuroImage*, *62*(4), 2190–200. https://doi.org/10.1016/j.neuroimage.2011.10.059

Leppänen, J. M., Moulson, M. C., Vogel-Farley, V. K., & Nelson, C. A. (2007). An ERP Study of Emotional Face Processing in the Adult and Infant Brain. *Child Development*, *78*(1), 232–245. https://doi.org/10.1111/j.1467-8624.2007.00994.x

Leslie, A. M. (1987). Pretense and representation: The origins of "theory of mind." *Psychological Review, 94*(4), 412–426. https://doi.org/10.1037/0033-295X.94.4.412

Levy, D., Ronemus, M., Yamrom, B., Lee, Y., Leotta, A., Kendall, J., … Wigler, M. (2011). Rare De Novo and Transmitted Copy-Number Variation in Autistic Spectrum Disorders. *Neuron, 70*(5), 886–897. https://doi.org/10.1016/j.neuron.2011.05.015

Li, T., Tian, Y., Li, Q., Chen, H., Lv, H., Xie, W., & Han, J. (2015). The Neurexin/N-Ethylmaleimide-sensitive Factor (NSF) Interaction Regulates Short Term Synaptic Depression. *The Journal of Biological Chemistry, 290*(29), 17656–67. https://doi.org/10.1074/jbc.M115.644583

Lichtenberg, N. T., Pennington, Z. T., Holley, S. M., Greenfield, V. Y., Cepeda, C., Levine, M. S., & Wassum, K. M. (2017). Basolateral amygdala to orbitofrontal cortex projections enable cue-triggered reward expectations. *The Journal of Neuroscience, 37*(35), 486–17. https://doi.org/10.1523/JNEUROSCI.0486-17.2017

Liebal, K., Colombi, C., Rogers, S. J., Warneken, F., & Tomasello, M. (2008). Helping and cooperation in children with autism. *Journal of Autism and Developmental Disorders, 38*(2), 224–38. https://doi.org/10.1007/s10803-007-0381-5

Lim, M. ., & Young, L. . (2004). Vasopressin-dependent neural circuits underlying pair bond formation in the monogamous prairie vole. *Neuroscience, 125*(1), 35–45. https://doi.org/10.1016/J.NEUROSCIENCE.2003.12.008

Lin, D., Boyle, M. P., Dollar, P., Lee, H., Lein, E. S., Perona, P., & Anderson, D. J. (2011). Functional identification of an aggression locus in the mouse hypothalamus. *Nature, 470*(7333), 221–226. https://doi.org/10.1038/nature09736

Lincoln, A. J., Searcy, Y. M., Jones, W., & Lord, C. (2007). Social Interaction Behaviors Discriminate Young Children With Autism and Williams Syndrome. *Journal of the American Academy of Child & Adolescent Psychiatry*, *46*(3), 323–331. https://doi.org/10.1097/CHI.0B013E31802B9522

Lindzey, G., Manosevitz, M., & Winston, H. (1966). Social dominance in the mouse. *Psychonomic Science*, *5*(11), 451–452. https://doi.org/10.3758/BF03331044

Lisman, J. (1997). Bursts as a unit of neural information: making unreliable synapses reliable. *Trends in Neurosciences*, *20*(1), 38–43. https://doi.org/10.1016/S0166-2236(96)10070-9

Liss, M., Saulnier, C., Fein, D., & Kinsbourne, M. (2006). Sensory and attention abnormalities in autistic spectrum disorders. *Autism*, *10*(2), 155–172. https://doi.org/10.1177/1362361306062021

Little, A. C., Jones, B. C., & DeBruine, L. M. (2011). The many faces of research on face perception. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *366*(1571), 1634–7. https://doi.org/10.1098/rstb.2010.0386

Litvin, Y., Blanchard, D. C., & Blanchard, R. J. (2007). Rat 22 kHz ultrasonic vocalizations as alarm cries. *Behavioural Brain Research*, *182*(2), 166–172. https://doi.org/10.1016/J.BBR.2006.11.038

Liu, C., Li, Y., Edwards, T. J., Kurniawan, N. D., Richards, L. J., & Jiang, T. (2016). Altered structural connectome in adolescent socially isolated mice. *NeuroImage*, *139*, 259–270. https://doi.org/10.1016/j.neuroimage.2016.06.037

Liu, J., Dietz, K., DeLoyht, J. M., Pedre, X., Kelkar, D., Kaur, J., … Casaccia, P. (2012). Impaired adult myelination in the prefrontal cortex of socially isolated mice. *Nature*

Neuroscience, *15*(12), 1621–1623. https://doi.org/10.1038/nn.3263

Liu, J., Dupree, J. L., Gacias, M., Frawley, R., Sikder, T., Naik, P., & Casaccia, P. (2016). Clemastine Enhances Myelination in the Prefrontal Cortex and Rescues Behavioral Changes in Socially Isolated Mice. *Journal of Neuroscience*, *36*(3), 957–962. https://doi.org/10.1523/JNEUROSCI.3608-15.2016

Lohmueller, K. E., Pearce, C. L., Pike, M., Lander, E. S., & Hirschhorn, J. N. (2003). Meta-analysis of genetic association studies supports a contribution of common variants to susceptibility to common disease. *Nature genetics*, 33(2), 177-182.

Löken, L. S., Wessberg, J., Morrison, I., McGlone, F., & Olausson, H. (2009). Coding of pleasant touch by unmyelinated afferents in humans. *Nature Neuroscience*, *12*(5), 547–548. https://doi.org/10.1038/nn.2312

Long, S. Y. (1972). Hair-nibbling and whisker-trimming as indicators of social hierarchy in mice. *Animal Behaviour*, *20*(1), 10–12. https://doi.org/10.1016/S0003-3472(72)80167-2

Losh, M., Adolphs, R., Poe, M. D., Couture, S., Penn, D., Baranek, G. T., & Piven, J. (2009). Neuropsychological Profile of Autism and the Broad Autism Phenotype. *Archives of General Psychiatry*, *66*(5), 518. https://doi.org/10.1001/archgenpsychiatry.2009.34

Loveland, K. A., Tunali-Kotoski, B., Chen, R., Brelsford, K. A., Ortegon, J., & Pearson, D. A. (1995). Intermodal perception of affect in persons with autism or Down syndrome. *Development and Psychopathology*, *7*(3), 409. https://doi.org/10.1017/S095457940000660X

Machado, C. J., & Bachevalier, J. (2006). The impact of selective amygdala, orbital frontal cortex, or hippocampal formation lesions on established social relationships in rhesus

monkeys (Macaca mulatta). *Behavioral Neuroscience*, *120*(4), 761–786. https://doi.org/10.1037/0735-7044.120.4.761

Mah, L., Arnold, M. C., & Grafman, J. (2004). Impairment of Social Perception Associated With Lesions of the Prefrontal Cortex. *American Journal of Psychiatry*, *161*(7), 1247–1255. https://doi.org/10.1176/appi.ajp.161.7.1247

Mah, L. W. Y., Arnold, M. C., & Grafman, J. (2005). Deficits in Social Knowledge Following Damage to Ventromedial Prefrontal Cortex. *The Journal of Neuropsychiatry and Clinical Neurosciences*, *17*(1), 66–74. https://doi.org/10.1176/jnp.17.1.66

Maher, B. (2008). Personal genomes: The case of the missing heritability. *Nature*, *456*(7218), 18–21. https://doi.org/10.1038/456018a

Makinodan, M., Ikawa, D., Miyamoto, Y., Yamauchi, J., Yamamuro, K., Yamashita, Y., … Kishimoto, T. (2016). Social isolation impairs remyelination in mice through modulation of IL-6. *The FASEB Journal*, *30*(12), 4267–4274. https://doi.org/10.1096/fj.201600537R

Makinodan, M., Rosen, K. M., Ito, S., & Corfas, G. (2012). A Critical Period for Social Experience-Dependent Oligodendrocyte Maturation and Myelination. *Science*, *337*(6100), 1357–1360. https://doi.org/10.1126/science.1220845

Mandel, D. R., Jusczyk, P. W., & Pisoni, D. B. (1995). Infants' Recognition of the Sound Patterns of Their Own Names. *Psychological Science*, *6*(5), 314–317. https://doi.org/10.1111/j.1467-9280.1995.tb00517.x

Manning, C., Charman, T., & Pellicano, E. (2013). Processing Slow and Fast Motion in Children With Autism Spectrum Conditions. *Autism Research*, *6*(6), 531–541.

https://doi.org/10.1002/aur.1309

Manolio, T. A., Collins, F. S., Cox, N. J., Goldstein, D. B., Hindorff, L. A., Hunter, D. J., … Visscher, P. M. (2009a). Finding the missing heritability of complex diseases. *Nature*, *461*(7265), 747–753. https://doi.org/10.1038/nature08494

Manolio, T. A., Collins, F. S., Cox, N. J., Goldstein, D. B., Hindorff, L. A., Hunter, D. J., … Visscher, P. M. (2009b). Finding the missing heritability of complex diseases. *Nature*, *461*(7265), 747–753. https://doi.org/10.1038/nature08494

Manuck, S. B., Flory, J. D., Ferrell, R. E., Mann, J. J., & Muldoon, M. F. (2000). A regulatory polymorphism of the monoamine oxidase-A gene may be associated with variability in aggression, impulsivity, and central nervous system serotonergic responsivity. *Psychiatry Research*, *95*(1), 9–23. https://doi.org/10.1016/S0165-1781(00)00162-1

Maravall, M., Stern, E. A., & Svoboda, K. (2004). Development of intrinsic properties and excitability of layer 2/3 pyramidal neurons during a critical period for sensory maps in rat barrel cortex. *Journal of Neurophysiology*, *92*(1), 144–56. https://doi.org/10.1152/jn.00598.2003

Marlin, B. J., Mitre, M., D'amour, J. A., Chao, M. V., & Froemke, R. C. (2015). Oxytocin enables maternal behaviour by balancing cortical inhibition. *Nature*, *520*(7548), 499–504. https://doi.org/10.1038/nature14402

Martens, M. A., Wilson, S. J., & Reutens, D. C. (2008). Research Review: Williams syndrome: a critical review of the cognitive, behavioral, and neuroanatomical phenotype. *Journal of Child Psychology and Psychiatry*, *49*(6), 576–608. https://doi.org/10.1111/j.1469-7610.2008.01887.x

Martineau, J., Roux, S., Adrien, J. L., Garreau, B., Barthélémy, C., & Lelord, G. (1992). Electrophysiological evidence of different abilities to form cross-modal associations in children with autistic behavior. *Electroencephalography and Clinical Neurophysiology*, *82*(1), 60–66. https://doi.org/10.1016/0013-4694(92)90183-I

Masciuch, S., & Kienapple, K. (1993). The Emergence of Jealousy in Children 4 Months to 7 Years of Age. *Journal of Social and Personal Relationships*, *10*(3), 421–435. https://doi.org/10.1177/0265407593103008

Mataix-Cols, D., An, S. K., Lawrence, N. S., Caseras, X., Speckens, A., Giampietro, V., … Phillips, M. L. (2008). Individual differences in disgust sensitivity modulate neural responses to aversive/disgusting stimuli. *European Journal of Neuroscience*, *27*(11), 3050–3058. https://doi.org/10.1111/j.1460-9568.2008.06311.x

Mateer, C. A., & Williams, D. (1991). Effects of frontal lobe injury in childhood. *Developmental Neuropsychology*, *7*(3), 359–376. https://doi.org/10.1080/87565649109540498

Matsuzawa, J., Matsui, M., Konishi, T., Noguchi, K., Gur, R. C., Bilker, W., & Miyawaki, T. (2001). Age-related Volumetric Changes of Brain Gray and White Matter in Healthy Infants and Children. *Cerebral Cortex, 11*(4), 335–342. https://doi.org/10.1093/cercor/11.4.335

Maynard, T. M., Sikich, L., Lieberman, J. A., & LaMantia, A.-S. (2001). Neural Development, Cell-Cell Signaling, and the "Two-Hit" Hypothesis of Schizophrenia. *Schizophrenia Bulletin*, *27*(3), 457–476. https://doi.org/10.1093/oxfordjournals.schbul.a006887

McAlonan, K., & Brown, V. J. (2003). Orbital prefrontal cortex mediates reversal learning

and not attentional set shifting in the rat. *Behavioural Brain Research*, *146*(1–2), 97–103. https://doi.org/10.1016/J.BBR.2003.09.019

McCauley, E., Kay, T., Ito, J., & Treder, R. (1987). The Turner Syndrome: Cognitive Deficits, Affective Discrimination, and Behavior Problems. *Child Development*, *58*(2), 464. https://doi.org/10.2307/1130523

McCormick, D. A., & Prince, D. A. (1986). Acetylcholine induces burst firing in thalamic reticular neurones by activating a potassium conductance. *Nature*, *319*(6052), 402–405. https://doi.org/10.1038/319402a0

Mcdonald, A. J. (1991). Organization of amygdaloid projections to the prefrontal cortex and associated striatum in the rat. *Neuroscience*, *44*(1), 1–14. https://doi.org/10.1016/0306-4522(91)90247-L

McDonnell, M. D., & Graham, B. P. (2017). Phase changes in neuronal postsynaptic spiking due to short term plasticity. *PLOS Computational Biology*, *13*(9), e1005634. https://doi.org/10.1371/journal.pcbi.1005634

McFarlane, H. G., Kusek, G. K., Yang, M., Phoenix, J. L., Bolivar, V. J., & Crawley, J. N. (2008). Autism-like behavioral phenotypes in BTBR T+tf/J mice. *Genes, Brain and Behavior*, *7*(2), 152–163. https://doi.org/10.1111/j.1601-183X.2007.00330.x

McGlone, F., Olausson, H., Boyle, J. A., Jones-Gotman, M., Dancer, C., Guest, S., & Essick, G. (2012). Touching and feeling: differences in pleasant touch processing between glabrous and hairy skin in humans. *European Journal of Neuroscience*, *35*(11), 1782–1788. https://doi.org/10.1111/j.1460-9568.2012.08092.x

McGrath, J., Saha, S., Chant, D., & Welham, J. (2008). Schizophrenia: A Concise Overview

of Incidence, Prevalence, and Mortality. *Epidemiologic Reviews*, *30*(1), 67–76. https://doi.org/10.1093/epirev/mxn001

McKay, B. M., Oh, M. M., & Disterhoft, J. F. (2013). Learning increases intrinsic excitability of hippocampal interneurons. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *33*(13), 5499–506. https://doi.org/10.1523/JNEUROSCI.4068-12.2013

McRae, K., Gross, J. J., Weber, J., Robertson, E. R., Sokol-Hessner, P., Ray, R. D., … Ochsner, K. N. (2012). The development of emotion regulation: an fMRI study of cognitive reappraisal in children, adolescents and young adults. *Social Cognitive and Affective Neuroscience*, *7*(1), 11–22. https://doi.org/10.1093/scan/nsr093

Meda, S. A., Pryweller, J. R., & Thornton-Wells, T. A. (2012). Regional Brain Differences in Cortical Thickness, Surface Area and Subcortical Volume in Individuals with Williams Syndrome. *PLoS ONE*, *7*(2), e31913. https://doi.org/10.1371/journal.pone.0031913

Meltzoff, A. N., & Moore, M. K. (1977). Imitation of facial and manual gestures by human neonates. *Science (New York, N.Y.)*, *198*(4312), 74–8. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/897687

Meltzoff, A. N., & Moore, M. K. (1983). Newborn Infants Imitate Adult Facial Gestures. *Child Development*, *54*(3), 702. https://doi.org/10.2307/1130058

Meyer-Lindenberg, A. (2008). Impact of prosocial neuropeptides on human brain function. *Progress in Brain Research*, *170*, 463–470. https://doi.org/10.1016/S0079-6123(08)00436-6

Meyer-Lindenberg, A., Hariri, A. R., Munoz, K. E., Mervis, C. B., Mattay, V. S., Morris, C.

A., & Berman, K. F. (2005). Neural correlates of genetically abnormal social cognition in Williams syndrome. *Nature Neuroscience*, *8*(8), 991–993. https://doi.org/10.1038/nn1494

Milshtein-Parush, H., Frere, S., Regev, L., Lahav, C., Benbenishty, A., Ben-Eliyahu, S., … Slutsky, I. (2017). Sensory Deprivation Triggers Synaptic and Intrinsic Plasticity in the Hippocampus. *Cerebral Cortex*, *27*(6), 3457–3470. https://doi.org/10.1093/cercor/bhx084

Mimura, M., Hoeft, F., Kato, M., Kobayashi, N., Sheau, K., Piggot, J., … Reiss, A. L. (2010). A preliminary study of orbitofrontal activation and hypersociability in Williams Syndrome. *Journal of Neurodevelopmental Disorders*, *2*(2), 93–98. https://doi.org/10.1007/s11689-009-9041-8

Missler, M., & Südhof, T. C. (1998). Neurexins: Three genes and 1001 products. *Trends in Genetics*, *14*(1), 20–26. https://doi.org/10.1016/S0168-9525(97)01324-3

Missler, M., Zhang, W., Rohlmann, A., Kattenstroth, G., Hammer, R. E., Gottmann, K., & Sudhof, T. C. (2003). [alpha]-Neurexins couple Ca2+ channels to synaptic vesicle exocytosis. *Nature*, *423*(6943), 939–949.

Missler, M., Zhang, W., Rohlmann, A., Kattenstroth, G., Hammer, R. E., Gottmann, K., & Südhof, T. C. (2003). α-Neurexins couple Ca2+ channels to synaptic vesicle exocytosis. *Nature*, *423*(6943), 939–948. https://doi.org/10.1038/nature01755

Misslin, R., & Ropartz, P. (1981). Responses in Mice To a Novel Object. *Behaviour*, *78*(3), 169–177. https://doi.org/10.1163/156853981X00301

Mitchell, K. J. (2011). The genetics of neurodevelopmental disease. *Current Opinion in*

Neurobiology, *21*(1), 197–203. https://doi.org/10.1016/J.CONB.2010.08.009

Miyata, J., Yamada, M., Namiki, C., Hirao, K., Saze, T., Fujiwara, H., … Murai, T. (2010). Reduced white matter integrity as a neural correlate of social cognition deficits in schizophrenia. *Schizophrenia Research*, *119*(1–3), 232–239. https://doi.org/10.1016/J.SCHRES.2009.12.038

Mizuno, A., Villalobos, M. E., Davies, M. M., Dahl, B. C., & Müller, R.-A. (2006). Partially enhanced thalamocortical functional connectivity in autism. *Brain Research*, *1104*(1), 160–174. https://doi.org/10.1016/J.BRAINRES.2006.05.064

Mohamed, S., Paulsen, J. S., O'Leary, D., Arndt, S., & Andreasen, N. (1999). Generalized Cognitive Deficits in Schizophrenia. *Archives of General Psychiatry*, *56*(8), 749. https://doi.org/10.1001/archpsyc.56.8.749

Moles, A., Costantini, F., Garbugino, L., Zanettini, C., & D'Amato, F. R. (2007). Ultrasonic vocalizations emitted during dyadic interactions in female mice: A possible index of sociability? *Behavioural Brain Research*, *182*(2), 223–230. https://doi.org/10.1016/j.bbr.2007.01.020

Moll, J., de Oliveira-Souza, R., Eslinger, P. J., Bramati, I. E., Mourão-Miranda, J., Andreiuolo, P. A., & Pessoa, L. (2002). The Neural Correlates of Moral Sensitivity: A Functional Magnetic Resonance Imaging Investigation of Basic and Moral Emotions. *Journal of Neuroscience*, *22*(7).

Moll, J., Zahn, R., de Oliveira-Souza, R., Krueger, F., & Grafman, J. (2005). Opinion: The neural basis of human moral cognition. *Nature Reviews Neuroscience*, *6*(10), 799–809. https://doi.org/10.1038/nrn1768

Mongillo, G., Barak, O., & Tsodyks, M. (2008). Synaptic theory of working memory. *Science (New York, N.Y.)*, *319*(5869), 1543–6. https://doi.org/10.1126/science.1150769

Moran, J. M., Young, L. L., Saxe, R., Lee, S. M., O'Young, D., Mavros, P. L., & Gabrieli, J. D. (2011). Impaired theory of mind for moral judgment in high-functioning autism. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(7), 2688–92. https://doi.org/10.1073/pnas.1011734108

Morecraft, R. J., Geula, C., & Mesulam, M.-M. (1992). Cytoarchitecture and neural afferents of orbitofrontal cortex in the brain of the monkey. *The Journal of Comparative Neurology*, *323*(3), 341–358. https://doi.org/10.1002/cne.903230304

Moretti, P., & Zoghbi, H. Y. (2006). MeCP2 dysfunction in Rett syndrome and related disorders. *Current Opinion in Genetics & Development*, *16*(3), 276–281. https://doi.org/10.1016/J.GDE.2006.04.009

Morgan, V. A., Leonard, H., Bourke, J., & Jablensky, A. (2008). Intellectual disability co-occurring with schizophrenia and other psychiatric illness: population-based study. *The British Journal of Psychiatry : The Journal of Mental Science*, *193*(5), 364–72. https://doi.org/10.1192/bjp.bp.107.044461

Morris, R. (1984). Developments of a water-maze procedure for studying spatial learning in the rat. *Journal of Neuroscience Methods*, *11*(1), 47–60. https://doi.org/10.1016/0165-0270(84)90007-4

Moy, S. S., Nadler, J. J., Young, N. B., Nonneman, R. J., Grossman, A. W., Murphy, D. L., … Lauder, J. M. (2009). Social approach in genetically engineered mouse lines relevant to autism. *Genes, Brain and Behavior*, *8*(2), 129–142. https://doi.org/10.1111/j.1601-183X.2008.00452.x

Munafò, M. R., Brown, S. M., & Hariri, A. R. (2008). Serotonin transporter (5-HTTLPR) genotype and amygdala activation: a meta-analysis. *Biological Psychiatry*, *63*(9), 852–7. https://doi.org/10.1016/j.biopsych.2007.08.016

Mundy, P., & Newell, L. (2007). Attention, Joint Attention, and Social Cognition. *Current Directions in Psychological Science*, *16*(5), 269–274. https://doi.org/10.1111/j.1467-8721.2007.00518.x

Mundy, P., Sigman, M., Ungerer, J., & Sherman, T. (1986). Defining the social deficits of autism: the contribution of non-verbal communication measures. *Journal of Child Psychology and Psychiatry*, *27*(5), 657–669. https://doi.org/10.1111/j.1469-7610.1986.tb00190.x

Nadler, J. J., Moy, S. S., Dold, G., Simmons, N., Perez, A., Young, N. B., … Crawley, J. N. (2004). Automated apparatus for quantitation of social approach behaviors in mice. *Genes, Brain and Behavior*, *3*(5), 303–314. https://doi.org/10.1111/j.1601-183X.2004.00071.x

Narr, K. L., Thompson, P. M., Sharma, T., Moussai, J., Cannestra, A. F., & Toga, A. W. (2000). Mapping Morphology of the Corpus Callosum in Schizophrenia. *Cerebral Cortex*, *10*(1), 40–49. https://doi.org/10.1093/cercor/10.1.40

Neher, E. (1998). Usefulness and limitations of linear approximations to the understanding of Ca++ signals. *Cell Calcium*, *24*(5–6), 345–357. https://doi.org/10.1016/S0143-4160(98)90058-6

Nelson, E. D., Kavalali, E. T., & Monteggia, L. M. (2006). MeCP2-Dependent Transcriptional Repression Regulates Excitatory Neurotransmission. *Current Biology*, *16*(7), 710–716. https://doi.org/10.1016/j.cub.2006.02.062

Nestler, E. J., & Hyman, S. E. (2010). Animal models of neuropsychiatric disorders. *Nature Neuroscience, 13*(10), 1161–9. https://doi.org/10.1038/nn.2647

Nilsson, S. R., Fejgin, K., Gastambide, F., Vogt, M. A., Kent, B. A., Nielsen, V., … Bussey, T. J. (2016). Assessing the Cognitive Translational Potential of a Mouse Model of the 22q11.2 Microdeletion Syndrome. *Cerebral Cortex, 26*(10), 3991–4003. https://doi.org/10.1093/cercor/bhw229

Nonneman, A. J., Voigt, J., & Kolb, B. E. (1974). Comparisons of behavioral effects of hippocampal and prefrontal cortex lesions in the rat. *Journal of Comparative and Physiological Psychology*, *87*(2), 249–260. https://doi.org/10.1037/h0036864

Noonan, M. P., Kolling, N., Walton, M. E., & Rushworth, M. F. S. (2012). Re-evaluating the role of the orbitofrontal cortex in reward and reinforcement. *European Journal of Neuroscience*, *35*(7), 997–1010. https://doi.org/10.1111/j.1460-9568.2012.08023.x

Nuechterlein, K. H., Barch, D. M., Gold, J. M., Goldberg, T. E., Green, M. F., & Heaton, R. K. (2004). Identification of separable cognitive factors in schizophrenia. *Schizophrenia research*, 72(1), 29-39.

Nyby, J. (1983). Ultrasonic vocalizations during sex behavior of male house mice (Mus musculus): A description. *Behavioral and Neural Biology*, *39*(1), 128–134. https://doi.org/10.1016/S0163-1047(83)90722-7

O'Connor, T. G., Bredenkamp, D., & Rutter, M. (1999). Attachment disturbances and disorders in children exposed to early severe deprivation. *Infant Mental Health Journal, 20*(1), 10–29. https://doi.org/10.1002/(SICI)1097-0355(199921)20:1<10::AID-IMHJ2>3.0.CO;2-S

O'Connor, V. M., Shamotienko, O., Grishin, E., & Betz, H. (1993). On the structure of the "synaptosecretosome" Evidence for a neurexin/synaptotagmin/syntaxin/Ca2+ channel complex. *FEBS Letters*, *326*(1–3), 255–260. https://doi.org/10.1016/0014-5793(93)81802-7

Oblak, A., Gibbs, T. T., & Blatt, G. J. (2009). Decreased GABA $_A$ receptors and benzodiazepine binding sites in the anterior cingulate cortex in autism. *Autism Research*, *2*(4), 205–219. https://doi.org/10.1002/aur.88

Olausson, H., Lamarre, Y., Backlund, H., Morin, C., Wallin, B. G., Starck, G., … Bushnell, M. C. (2002). Unmyelinated tactile afferents signal touch and project to insular cortex. *Nature Neuroscience*, *5*(9), 900–904. https://doi.org/10.1038/nn896

Olausson, H., Wessberg, J., Morrison, I., McGlone, F., & Vallbo, Å. (2010). The neurophysiology of unmyelinated tactile afferents. *Neuroscience & Biobehavioral Reviews*, *34*(2), 185–191. https://doi.org/10.1016/J.NEUBIOREV.2008.09.011

Olazábal, D. E., & Young, L. J. (2006). Oxytocin receptors in the nucleus accumbens facilitate "spontaneous" maternal behavior in adult female prairie voles. *Neuroscience*, *141*(2), 559–568. https://doi.org/10.1016/J.NEUROSCIENCE.2006.04.017

Olson, I. R., Von Der Heide, R. J., Alm, K. H., & Vyas, G. (2015). Development of the uncinate fasciculus: Implications for theory and developmental disorders. *Developmental Cognitive Neuroscience*, *14*, 50–61. https://doi.org/10.1016/j.dcn.2015.06.003

Ongur, D., & Price, J. L. (2000). The Organization of Networks within the Orbital and Medial Prefrontal Cortex of Rats, Monkeys and Humans. *Cerebral Cortex*, *10*(3), 206–219. https://doi.org/10.1093/cercor/10.3.206

Orefice, L. L., Zimmerman, A. L., Chirila, A. M., Sleboda, S. J., Head, J. P., & Ginty, D. D. (2016). Peripheral Mechanosensory Neuron Dysfunction Underlies Tactile and Behavioral Deficits in Mouse Models of ASDs. *Cell*, *166*(2), 299–313. https://doi.org/10.1016/J.CELL.2016.05.033

Orozco-Cabal, L., Pollandt, S., Liu, J., Vergara, L., Shinnick-Gallagher, P., & Gallagher, J. P. (2006). A novel rat medial prefrontal cortical slice preparation to investigate synaptic transmission from amygdala to layer V prelimbic pyramidal neurons. *Journal of Neuroscience Methods*, *151*(2), 148–158. https://doi.org/10.1016/j.jneumeth.2005.07.002

Osterling, J. A., Dawson, G., & Munson, J. A. (2002). Early recognition of 1-year-old infants with autism spectrum disorder versus mental retardation. *Development and Psychopathology*, *14*(2), 239–51.

Ozonoff, S., & McEvoy, R. E. (1994). A longitudinal study of executive function and theory of mind development in autism. *Development and Psychopathology*, *6*(3), 415. https://doi.org/10.1017/S0954579400006027

Ozonoff, S., Pennington, B. F., & Rogers, S. J. (1991). Executive Function Deficits in High-Functioning Autistic Individuals: Relationship to Theory of Mind. *Journal of Child Psychology and Psychiatry*, *32*(7), 1081–1105. https://doi.org/10.1111/j.1469-7610.1991.tb00351.x

Pachou, E., Vourkas, M., Simos, P., Smit, D., Stam, C. J., Tsirka, V., & Micheloyannis, S. (2008). Working Memory in Schizophrenia: An EEG Study Using Power Spectrum and Coherence Analysis to Estimate Cortical Activation and Network Behavior. *Brain Topography*, *21*(2), 128–137. https://doi.org/10.1007/s10548-008-0062-5

Paillère-Martinot, M.-L., Caclin, A., Artiges, E., Poline, J.-B., Joliot, M., Mallet, L., … Martinot, J.-L. (2001). Cerebral gray and white matter reductions and clinical correlates in patients with early onset schizophrenia. *Schizophrenia Research*, *50*(1–2), 19–26. https://doi.org/10.1016/S0920-9964(00)00137-7

Panksepp, J. (1981). The ontogeny of play in rats. *Developmental Psychobiology*, *14*(4), 327–332. https://doi.org/10.1002/dev.420140405

Papcun, G., Kreiman, J., & Davis, A. (1989). Long-term memory for unfamiliar voices. *The Journal of the Acoustical Society of America*, *85*(2), 913–25.

Parkinson, C., & Wheatley, T. (2014). Relating Anatomical and Social Connectivity: White Matter Microstructure Predicts Emotional Empathy. *Cerebral Cortex*, *24*(3), 614–625. https://doi.org/10.1093/cercor/bhs347

Pascalis, O., de Schonen, S., Morton, J., Deruelle, C., & Fabre-Grenet, M. (1995). Mother's face recognition by neonates: A replication and an extension. *Infant Behavior and Development*, *18*(1), 79–85. https://doi.org/10.1016/0163-6383(95)90009-8

Passamonti, L., Crockett, M. J., Apergis-Schoute, A. M., Clark, L., Rowe, J. B., Calder, A. J., & Robbins, T. W. (2012). Effects of Acute Tryptophan Depletion on Prefrontal-Amygdala Connectivity While Viewing Facial Signals of Aggression. *Biological Psychiatry*, *71*(1), 36–43. https://doi.org/10.1016/J.BIOPSYCH.2011.07.033

Paus, T. (2010). Growth of white matter in the adolescent brain: Myelin or axon? *Brain and Cognition*, *72*(1), 26–35. https://doi.org/10.1016/j.bandc.2009.06.002

Pedersen, C. A., Prange, A. J., & Jr. (1979). Induction of maternal behavior in virgin rats after intracerebroventricular administration of oxytocin. *Proceedings of the National*

Academy of Sciences of the United States of America, *76*(12), 6661–5. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/293752

Pellicano, E. (2007). Links between theory of mind and executive function in young children with autism: Clues to developmental primacy. *Developmental Psychology*, *43*(4), 974–990. https://doi.org/10.1037/0012-1649.43.4.974

Pellis, S. M., Hastings, E., Shimizu, T., Kamitakahara, H., Komorowska, J., Forgie, M. L., & Kolb, B. (2006). The effects of orbital frontal cortex damage on the modulation of defensive responses by rats in playful and nonplayful social contexts. *Behavioral Neuroscience*, *120*(1), 72–84. https://doi.org/10.1037/0735-7044.120.1.72

Pellis, S. M., & McKenna, M. (1995). What do rats find rewarding in play fighting? — an analysis using drug-induced non-playful partners. *Behavioural Brain Research*, *68*(1), 65–73. https://doi.org/10.1016/0166-4328(94)00161-8

Pellis, S. M., & Pellis, V. C. (1987). Play-fighting differs from serious fighting in both target of attack and tactics of fighting in the laboratory ratRattus norvegicus. *Aggressive Behavior*, *13*(4), 227–242. https://doi.org/10.1002/1098-2337(1987)13:4<227::AID-AB2480130406>3.0.CO;2-C

Pellis, S. M., & Pellis, V. C. (1991). Role reversal changes during the ontogeny of play fighting in male rats: Attack vs. defense. *Aggressive Behavior*, *17*(3), 179–189. https://doi.org/10.1002/1098-2337(1991)17:3<179::AID-AB2480170306>3.0.CO;2-Q

Pelphrey, K. A., Viola, R. J., & McCarthy, G. (2004). When Strangers Pass. *Psychological Science*, *15*(9), 598–603. https://doi.org/10.1111/j.0956-7976.2004.00726.x

Peñagarikano, O., Abrahams, B. S., Herman, E. I., Winden, K. D., Gdalyahu, A., Dong, H.,

… Geschwind, D. H. (2011a). Absence of CNTNAP2 Leads to Epilepsy, Neuronal Migration Abnormalities, and Core Autism-Related Deficits. *Cell*, *147*(1), 235–246. https://doi.org/10.1016/j.cell.2011.08.040

Penn, D. L., Corrigan, P. W., Bentall, R. P., Racenstein, J. M., & Newman, L. (1997). Social Cognition in Schizophrenia. *Psychological Bulletin Bentall*, *121*(1), 114–132.

Penn, D. L., Sanna, L. J., & Roberts, D. L. (2008). Social Cognition in Schizophrenia: An Overview. *Schizophrenia Bulletin*, *34*(3), 408–411. https://doi.org/10.1093/schbul/sbn014

Perner, J., Frith, U., Leslie, A. M., & Leekam, S. R. (1989). Exploration of the Autistic Child's Theory of Mind: Knowledge, Belief, and Communication. *Child Development*, *60*(3), 689. https://doi.org/10.2307/1130734

Perrett, D. I., Rolls, E. T., & Caan, W. (1982). Visual neurones responsive to faces in the monkey temporal cortex. *Experimental Brain Research*, *47*(3), 329–342. https://doi.org/10.1007/BF00239352

Petanjek, Z., Judaš, M., Šimic, G., Rasin, M. R., Uylings, H. B. M., Rakic, P., & Kostovic, I. (2011). Extraordinary neoteny of synaptic spines in the human prefrontal cortex. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(32), 13281–6. https://doi.org/10.1073/pnas.1105108108

Peykov, S., Berkel, S., Schoen, M., Weiss, K., Degenhardt, F., Strohmaier, J., … Rappold, G. A. (2015). Identification and functional characterization of rare SHANK2 variants in schizophrenia. *Molecular Psychiatry*, *20*(12), 1489–1498. https://doi.org/10.1038/mp.2014.172

Pinkham, A. E., Penn, D. L., Green, M. F., Buck, B., Healey, K., & Harvey, P. D. (2014). The Social Cognition Psychometric Evaluation Study: Results of the Expert Survey and RAND Panel. *Schizophrenia Bulletin*, *40*(4), 813–823. https://doi.org/10.1093/schbul/sbt081

Pinquart, M., & Sörensen, S. (2000). Influences of socioeconomic status, social network, and competence on subjective well-being in later life: A meta-analysis. *Psychology and Aging*, *15*(2), 187–224. https://doi.org/10.1037/0882-7974.15.2.187

Pinto, D., Delaby, E., Merico, D., Barbosa, M., Merikangas, A., Klei, L., … Scherer, S. W. (2014). Convergence of Genes and Cellular Pathways Dysregulated in Autism Spectrum Disorders. *The American Journal of Human Genetics*, *94*(5), 677–694. https://doi.org/10.1016/J.AJHG.2014.03.018

Plesa-Skwerer, D., Faja, S., Schofield, C., Verbalis, A., & Tager-Flusberg, H. (2006). Perceiving facial and vocal expressions of emotion in individuals with Williams syndrome. *American Journal of Mental Retardation : AJMR*, *111*(1), 15–26. https://doi.org/10.1352/0895-8017(2006)111[15:PFAVEO]2.0.CO;2

Pocklington, A. J., O'Donovan, M., & Owen, M. J. (2014). The synapse in schizophrenia. *European Journal of Neuroscience*, *39*(7), 1059–1067. https://doi.org/10.1111/ejn.12489

Porter, M. A., Coltheart, M., & Langdon, R. (2008). Theory of Mind in Williams Syndrome Assessed Using a Nonverbal Task. *Journal of Autism and Developmental Disorders*, *38*(5), 806–814. https://doi.org/10.1007/s10803-007-0447-4

Potegal, M., & Einon, D. (1989). Aggressive behaviors in adult rats deprived of playfighting experience as juveniles. *Developmental Psychobiology*, *22*(2), 159–172. https://doi.org/10.1002/dev.420220206

Preuss, T. M. (1995). Do Rats Have Prefrontal Cortex? The Rose-Woolsey-Akert Program Reconsidered. *Journal of Cognitive Neuroscience*, *7*(1), 1–24. https://doi.org/10.1162/jocn.1995.7.1.1

Pritchard, J. K. (2001). Are Rare Variants Responsible for Susceptibility to Complex Diseases? *The American Journal of Human Genetics*, *69*(1), 124–137. https://doi.org/10.1086/321272

Purcell, A. E., Jeon, O. H., Zimmerman, A. W., Blue, M. E., & Pevsner, J. (2001). Postmortem brain abnormalities of the glutamate neurotransmitter system in autism. *Neurology*, *57*(9), 1618–28. https://doi.org/10.1212/WNL.57.9.1618

Purcell, D. G., & Stewart, A. L. (1988). The face-detection effect: Configuration enhances detection. *Perception & Psychophysics*, *43*(4), 355–366. https://doi.org/10.3758/BF03208806

Qiu, S., Anderson, C. T., Levitt, P., & Shepherd, G. M. G. (2011). Circuit-Specific Intracortical Hyperconnectivity in Mice with Deletion of the Autism-Associated Met Receptor Tyrosine Kinase. *Journal of Neuroscience*, *31*(15), 5855–5864. https://doi.org/10.1523/JNEUROSCI.6569-10.2011

Radoeva, P. D., Coman, I. L., Antshel, K. M., Fremont, W., McCarthy, C. S., Kotkar, A., … Kates, W. R. (2012). Atlas-based white matter analysis in individuals with velo-cardio-facial syndrome (22q11.2 deletion syndrome) and unaffected siblings. *Behavioral and Brain Functions*, *8*(1), 38. https://doi.org/10.1186/1744-9081-8-38

Radua, J., Via, E., Catani, M., & Mataix-Cols, D. (2011). Voxel-based meta-analysis of regional white-matter volume differences in autism spectrum disorder versus healthy controls. *Psychological Medicine*, *41*(7), 1539–1550.

https://doi.org/10.1017/S0033291710002187

Rainer, G., & Miller, E. K. (2002). Timecourse of object-related neural activity in the primate prefrontal cortex during a short-term memory task. *European Journal of Neuroscience*, *15*(7), 1244–1254. https://doi.org/10.1046/j.1460-9568.2002.01958.x

Rajkovich, K. E., Loerwald, K. W., Hale, C. F., Hess, C. T., Gibson, J. R., & Huber, K. M. (2017). Experience-Dependent and Differential Regulation of Local and Long-Range Excitatory Neocortical Circuits by Postsynaptic Mef2c. *Neuron*, *93*(1), 48–56. https://doi.org/10.1016/J.NEURON.2016.11.022

Ramirez-Lugo, L., Penas-Rincon, A., Angeles-Duran, S., & Sotres-Bayon, F. (2016). Choice Behavior Guided by Learned, But Not Innate, Taste Aversion Recruits the Orbitofrontal Cortex. *Journal of Neuroscience*, *36*(41), 10574–10583. https://doi.org/10.1523/JNEUROSCI.0796-16.2016

Ray, E., & Heyes, C. (2011). Imitation in infancy: the wealth of the stimulus. *Developmental Science*, *14*(1), 92–105. https://doi.org/10.1111/j.1467-7687.2010.00961.x

Reader, S. M., & Laland, K. N. (2002). Social intelligence, innovation, and enhanced brain size in primates. *Proceedings of the National Academy of Sciences of the United States of America*, *99*(7), 4436–41. https://doi.org/10.1073/pnas.062041299

Reddy, V., & Mireault, G. (2015). Teasing and clowning in infancy. *Current Biology*, *25*(1), R20–R23. https://doi.org/10.1016/J.CUB.2014.09.021

Reep, R. L., Corwin, J. V., & King, V. (1996). Neuronal connections of orbital cortex in rats: topography of cortical and thalamic afferents. *Experimental Brain Research*, *111*(2), 215–232. https://doi.org/10.1007/BF00227299

Reich, D. E., & Lander, E. S. (2001). On the allelic spectrum of human disease. *Trends in Genetics : TIG*, *17*(9), 502–10.

Rempel-Clower, N. L., & Barbas, H. (1998). Topographic organization of connections between the hypothalamus and prefrontal cortex in the rhesus monkey. *The Journal of Comparative Neurology*, *398*(3), 393–419. https://doi.org/10.1002/(SICI)1096-9861(19980831)398:3<393::AID-CNE7>3.0.CO;2-V

Reyes, A., & Sakmann, B. (1999). Developmental Switch in the Short-Term Modification of Unitary EPSPs Evoked in Layer 2/3 and Layer 5 Pyramidal Neurons of Rat Neocortex. *Journal of Neuroscience*, *19*(10).

Rhee, S. H., & Waldman, I. D. (2002). Genetic and environmental influences on antisocial behavior: a meta-analysis of twin and adoption studies. *Psychological Bulletin*, *128*(3), 490–529.

Riby, D., & Hancock, P. J. B. (2009). Looking at movies and cartoons: eye-tracking evidence from Williams syndrome and autism. *Journal of Intellectual Disability Research*, *53*(2), 169–181. https://doi.org/10.1111/j.1365-2788.2008.01142.x

Riby, D. M., & Hancock, P. J. B. (2008). Viewing it differently: Social scene perception in Williams syndrome and Autism. *Neuropsychologia*, *46*(11), 2855–2860. https://doi.org/10.1016/J.NEUROPSYCHOLOGIA.2008.05.003

Riby, D. M., & Hancock, P. J. B. (2009). Do Faces Capture the Attention of Individuals with Williams Syndrome or Autism? Evidence from Tracking Eye Movements. *Journal of Autism and Developmental Disorders*, *39*(3), 421–431. https://doi.org/10.1007/s10803-008-0641-z

Rinaldi, T., Silberberg, G., & Markram, H. (2008). Hyperconnectivity of Local Neocortical Microcircuitry Induced by Prenatal Exposure to Valproic Acid. *Cerebral Cortex*, *18*(4), 763–770. https://doi.org/10.1093/cercor/bhm117

Ripke, S., Neale, B. M., Corvin, A., Walters, J. T. R., Farh, K.-H., Holmans, P. A., … O'Donovan, M. C. (2014). Biological insights from 108 schizophrenia-associated genetic loci. *Nature*, *511*(7510), 421–427. https://doi.org/10.1038/nature13595

Rizzoli, S. O., & Betz, W. J. (2005). Synaptic vesicle pools. *Nature Reviews Neuroscience*, *6*(1), 57–69. https://doi.org/10.1038/nrn1583

Robertson, C. E., & Baron-Cohen, S. (2017). Sensory perception in autism. *Nature Reviews Neuroscience*. https://doi.org/10.1038/nrn.2017.112

Rodríguez-Arias, M., Navarrete, F., Blanco-Gandia, M. C., Arenas, M. C., Aguilar, M. A., Bartoll-Andrés, A., … Manzanares, J. (2015). Role of CB2 receptors in social and aggressive behavior in male mice. *Psychopharmacology*, *232*(16), 3019–3031. https://doi.org/10.1007/s00213-015-3939-5

Rogers, S. J., Hepburn, S., & Wehner, E. (2003). Parent Reports of Sensory Symptoms in Toddlers with Autism and Those with Other Developmental Disorders. *Journal of Autism and Developmental Disorders*, *33*(6), 631–642. https://doi.org/10.1023/B:JADD.0000006000.38991.a7

Rogers, S. J., & Pennington, B. F. (1991). A theoretical approach to the deficits in infantile autism. *Development and Psychopathology*, *3*(2), 137–162. https://doi.org/10.1017/S0954579400005204

Rolls, E. T., & Baylis, L. L. (1994a). Gustatory, olfactory, and visual convergence within the

primate orbitofrontal cortex. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *14*(9), 5437–52.

Rolls, E. T., O'Doherty, J., Kringelbach, M. L., Francis, S., Bowtell, R., & McGlone, F. (2003). Representations of Pleasant and Painful Touch in the Human Orbitofrontal and Cingulate Cortices. *Cerebral Cortex*, *13*(3), 308–317. https://doi.org/10.1093/cercor/13.3.308

Rolls, E. T., O'Doherty, J., Kringelbach, M. L., Francis, S., Bowtell, R., & McGlone, F. (2003). Representations of pleasant and painful touch in the human orbitofrontal and cingulate cortices. *Cerebral Cortex (New York, N.Y. : 1991)*, *13*(3), 308–17.

Rolls, E. T., Sienkiewicz, Z. J., & Yaxley, S. (1989). Hunger Modulates the Responses to Gustatory Stimuli of Single Neurons in the Caudolateral Orbitofrontal Cortex of the Macaque Monkey. *European Journal of Neuroscience*, *1*(1), 53–60. https://doi.org/10.1111/j.1460-9568.1989.tb00774.x

Romanski, L. M., Tian, B., Fritz, J., Mishkin, M., Goldman-Rakic, P. S., & Rauschecker, J. P. (1999). Dual streams of auditory afferents target multiple domains in the primate prefrontal cortex. *Nature Neuroscience*, *2*(12), 1131–1136. https://doi.org/10.1038/16056

Ronald, A., Happé, F., Bolton, P., Butcher, L. M., Price, T. S., Wheelwright, S., … Plomin, R. (2006). Genetic Heterogeneity Between the Three Components of the Autism Spectrum: A Twin Study. *Journal of the American Academy of Child & Adolescent Psychiatry*, *45*(6), 691–699. https://doi.org/10.1097/01.CHI.0000215325.13058.9D

Ronald, A., Happe, F., & Plomin, R. (2005). The genetic relationship between individual differences in social and nonsocial behaviours characteristic of autism. *Developmental*

Science, *8*(5), 444–458. https://doi.org/10.1111/j.1467-7687.2005.00433.x

Ronald, A., & Hoekstra, R. A. (2011). Autism spectrum disorders and autistic traits: A decade of new twin studies. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*, *156*(3), 255–274. https://doi.org/10.1002/ajmg.b.31159

Rose, G. J., & Fortune, E. S. (1999). Frequency-dependent PSP depression contributes to low-pass temporal filtering in Eigenmannia. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *19*(17), 7629–39.

Rosner, B. A., Hodapp, R. M., Fidler, D. J., Sagun, J. N., & Dykens, E. M. (2004). Social Competence in Persons with Prader-Willi, Williams and Down's Syndromes. *Journal of Applied Research in Intellectual Disabilities*, *17*(3), 209–217. https://doi.org/10.1111/j.1468-3148.2004.00200.x

Ross, R. S., McGaughy, J., & Eichenbaum, H. (2005). Acetylcholine in the orbitofrontal cortex is necessary for the acquisition of a socially transmitted food preference. *Learning & Memory (Cold Spring Harbor, N.Y.)*, *12*(3), 302–6. https://doi.org/10.1101/lm.91605

Rozov, A., & Burnashev, N. (1999). Polyamine-dependent facilitation of postsynaptic AMPA receptors counteracts paired-pulse depression. *Nature*, *401*(6753), 594–598. https://doi.org/10.1038/44151

Rozov, A., Burnashev, N., Sakmann, B., & Neher, E. (2001). Transmitter release modulation by intracellular Ca^{2+} buffers in facilitating and depressing nerve terminals of pyramidal cells in layer 2/3 of the rat neocortex indicates a target cell-specific difference in presynaptic calcium dynamics. *The Journal of Physiology*, *531*(3), 807–826. https://doi.org/10.1111/j.1469-7793.2001.0807h.x

Rubenstein, J. L. R., & Merzenich, M. M. (2003). Model of autism: increased ratio of excitation/inhibition in key neural systems. *Genes, Brain and Behavior*, *2*(5), 255–267. https://doi.org/10.1034/j.1601-183X.2003.00037.x

Rudebeck, P. H., & Murray, E. A. (2014). The Orbitofrontal Oracle: Cortical Mechanisms for the Prediction and Evaluation of Specific Behavioral Outcomes. *Neuron*, *84*(6), 1143–1156. https://doi.org/10.1016/J.NEURON.2014.10.049

Rudebeck, P. H., Walton, M. E., Millette, B. H. P., Shirley, E., Rushworth, M. F. S., & Bannerman, D. M. (2007). Distinct contributions of frontal areas to emotion and social behaviour in the rat. *European Journal of Neuroscience*, *26*(8), 2315–2326. https://doi.org/10.1111/j.1460-9568.2007.05844.x

Rujescu, D., Ingason, A., Cichon, S., Pietiläinen, O. P. H., Barnes, M. R., Toulopoulou, T., … Collier, D. A. (2009). Disruption of the neurexin 1 gene is associated with schizophrenia. *Human Molecular Genetics*, *18*(5), 988–996. https://doi.org/10.1093/hmg/ddn351

Rutter, M. (1970). Autistic children: infancy to adulthood. *Seminars in Psychiatry*, *2*(4), 435–50.

Rutter, M. (1983). Cognitive deficits in the pathogenesis of autism. *Journal of Child Psychology and Psychiatry*, *24*(4), 513–531. https://doi.org/10.1111/j.1469-7610.1983.tb00129.x

Ryan, N. P., Anderson, V., Godfrey, C., Beauchamp, M. H., Coleman, L., Eren, S., … Catroppa, C. (2014). Predictors of Very-Long-Term Sociocognitive Function after Pediatric Traumatic Brain Injury: Evidence for the Vulnerability of the Immature "Social Brain." *Journal of Neurotrauma*, *31*(7), 649–657.

https://doi.org/10.1089/neu.2013.3153

Saez, R. A., Saez, A., Paton, J. J., Lau, B., & Salzman, C. D. (2017). Distinct Roles for the Amygdala and Orbitofrontal Cortex in Representing the Relative Amount of Expected Reward. *Neuron*, *95*(1), 70–77.e3. https://doi.org/10.1016/j.neuron.2017.06.012

Sala, M., Braida, D., Lentini, D., Busnelli, M., Bulgheroni, E., Capurro, V., … Chini, B. (2011). Pharmacologic Rescue of Impaired Cognitive Flexibility, Social Deficits, Increased Aggression, and Seizure Susceptibility in Oxytocin Receptor Null Mice: A Neurobehavioral Model of Autism. *Biological Psychiatry*, *69*(9), 875–882. https://doi.org/10.1016/J.BIOPSYCH.2010.12.022

Samson, D., Apperly, I. A., Chiavarino, C., & Humphreys, G. W. (2004). Left temporoparietal junction is necessary for representing someone else's belief. *Nature Neuroscience*, *7*(5), 499–500. https://doi.org/10.1038/nn1223

Sanders, S. J., Ercan-Sencicek, A. G., Hus, V., Luo, R., Murtha, M. T., Moreno-De-Luca, D., … State, M. W. (2011). Multiple Recurrent De Novo CNVs, Including Duplications of the 7q11.23 Williams Syndrome Region, Are Strongly Associated with Autism. *Neuron*, *70*(5), 863–885. https://doi.org/10.1016/J.NEURON.2011.05.002

Sandin, S., Lichtenstein, P., Kuja-Halkola, R., Larsson, H., Hultman, C. M., & Reichenberg, A. (2014). The Familial Risk of Autism. *JAMA*, *311*(17), 1770. https://doi.org/10.1001/jama.2014.4144

Sara, Y., Virmani, T., Deák, F., Liu, X., & Kavalali, E. T. (2005). An Isolated Pool of Vesicles Recycles at Rest and Drives Spontaneous Neurotransmission. *Neuron*, *45*(4), 563–573. https://doi.org/10.1016/j.neuron.2004.12.056

Sato, A., Kasai, S., Kobayashi, T., Takamatsu, Y., Hino, O., Ikeda, K., & Mizuguchi, M. (2012). Rapamycin reverses impaired social interaction in mouse models of tuberous sclerosis complex. *Nature Communications*, *3*, 1292. https://doi.org/10.1038/ncomms2295

Savaskan, E., Ehrhardt, R., Schulz, A., Walter, M., & Schächinger, H. (2008). Post-learning intranasal oxytocin modulates human memory for facial identity. *Psychoneuroendocrinology*, *33*(3), 368–374. https://doi.org/10.1016/J.PSYNEUEN.2007.12.004

Saver, J. L., & Damasio, A. R. (1991). Preserved access and processing of social knowledge in a patient with acquired sociopathy due to ventromedial frontal damage. *Neuropsychologia*, *29*(12), 1241–1249. https://doi.org/10.1016/0028-3932(91)90037-9

Saxe, R. (2006). Uniquely human social cognition. *Current Opinion in Neurobiology*, *16*(2), 235–239. https://doi.org/10.1016/J.CONB.2006.03.001

Saxe, R., Xiao, D.-K., Kovacs, G., Perrett, D. ., & Kanwisher, N. (2004). A region of right posterior superior temporal sulcus responds to observed intentional actions. *Neuropsychologia*, *42*(11), 1435–1446. https://doi.org/10.1016/J.NEUROPSYCHOLOGIA.2004.04.015

Saygin, A. P. (2007). Superior temporal and premotor brain areas necessary for biological motion perception. *Brain*, *130*(9), 2452–2461. https://doi.org/10.1093/brain/awm162

Scaife, M., & Bruner, J. S. (1975). The capacity for joint visual attention in the infant. *Nature*, *253*(5489), 265–266. https://doi.org/10.1038/253265a0

Scattoni, M. L., Crawley, J., & Ricceri, L. (2009). Ultrasonic vocalizations: A tool for

behavioural phenotyping of mouse models of neurodevelopmental disorders. *Neuroscience & Biobehavioral Reviews*, *33*(4), 508–515. https://doi.org/10.1016/J.NEUBIOREV.2008.08.003

Scattoni, M. L., Ricceri, L., & Crawley, J. N. (2011). Unusual repertoire of vocalizations in adult BTBR T+tf/J mice during three types of social encounters. *Genes, Brain and Behavior*, *10*(1), 44–56. https://doi.org/10.1111/j.1601-183X.2010.00623.x

Schaaf, C. P., Boone, P. M., Sampath, S., Williams, C., Bader, P. I., Mueller, J. M., … Cheung, S. W. (2012). Phenotypic spectrum and genotype–phenotype correlations of NRXN1 exon deletions. *European Journal of Human Genetics*, *20*(12), 1240–1247. https://doi.org/10.1038/ejhg.2012.95

Schaer, M., Ottet, M.-C., Scariati, E., Dukes, D., Franchini, M., Eliez, S., & Glaser, B. (2013). Decreased frontal gyrification correlates with altered connectivity in children with autism. *Frontiers in Human Neuroscience*, *7*, 750. https://doi.org/10.3389/fnhum.2013.00750

Schalkwyk, L. C., Fernandes, C., Nash, M. W., Kurrikoff, K., Vasar, E., & Kõks, S. (2007). Interpretation of knockout experiments: the congenic footprint. *Genes, Brain and Behavior*, *6*(3), 299–303. https://doi.org/10.1111/j.1601-183X.2007.00304.x

Scherer, K. R., Banse, R., Wallbott, H. G., & Goldbeck, T. (1991). Vocal cues in emotion encoding and decoding. *Motivation and Emotion*, *15*(2), 123–148. https://doi.org/10.1007/BF00995674

Schmidt, L., Skvortsova, V., Kullen, C., Weber, B., & Plassmann, H. (2017). How context alters value: The brain's valuation and affective regulation system link price cues to experienced taste pleasantness. *Scientific Reports*, *7*(1), 8098.

https://doi.org/10.1038/s41598-017-08080-0

Schoch, S., Deák, F., Königstorfer, A., Mozhayeva, M., Sara, Y., Südhof, T. C., & Kavalali, E. T. (2001). SNARE function analyzed in synaptobrevin/VAMP knockout mice. *Science (New York, N.Y.)*, *294*(5544), 1117–22. https://doi.org/10.1126/science.1064335

Schoenbaum, G., Chiba, A. A., & Gallagher, M. (1999). Neural encoding in orbitofrontal cortex and basolateral amygdala during olfactory discrimination learning. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *19*(5), 1876–84.

Schoenbaum, G., & Eichenbaum, H. (1995). Information coding in the rodent prefrontal cortex. I. Single-neuron activity in orbitofrontal cortex compared with that in pyriform cortex. *Journal of Neurophysiology*, *74*(2), 733–50.

Schoenbaum, G., Roesch, M. R., Stalnaker, T. A., & Takahashi, Y. K. (2009). A new perspective on the role of the orbitofrontal cortex in adaptive behaviour. *Nature Reviews. Neuroscience*, *10*(12), 885–92. https://doi.org/10.1038/nrn2753

Schoenbaum, G., Setlow, B., Saddoris, M. P., & Gallagher, M. (2003). Encoding Predicted Outcome and Acquired Value in Orbitofrontal Cortex during Cue Sampling Depends upon Input from Basolateral Amygdala. *Neuron*, *39*(5), 855–867. https://doi.org/10.1016/S0896-6273(03)00474-4

Schultz, R. T., Grelotti, D. J., Klin, A., Kleinman, J., Van der Gaag, C., Marois, R., & Skudlarski, P. (2003). The role of the fusiform face area in social cognition: implications for the pathobiology of autism. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *358*(1430), 415–27. https://doi.org/10.1098/rstb.2002.1208

Sebat, J., Lakshmi, B., Malhotra, D., Troge, J., Lese-Martin, C., Walsh, T., … Wigler, M. (2007). Strong association of de novo copy number mutations with autism. *Science (New York, N.Y.), 316*(5823), 445–9. https://doi.org/10.1126/science.1138659

Sehgal, M., Ehlers, V. L., & Moyer, J. R. (2014). Learning enhances intrinsic excitability in a subset of lateral amygdala neurons. *Learning & Memory (Cold Spring Harbor, N.Y.), 21*(3), 161–70. https://doi.org/10.1101/lm.032730.113

Senju, A., Johnson, M. H., & Csibra, G. (2006). The development and neural basis of referential gaze perception. *Social Neuroscience, 1*(3–4), 220–234. https://doi.org/10.1080/17470910600989797

Senju, A., Southgate, V., White, S., & Frith, U. (2009). Mindblind eyes: an absence of spontaneous theory of mind in Asperger syndrome. *Science (New York, N.Y.), 325*(5942), 883–5. https://doi.org/10.1126/science.1176170

Sergerie, K., Chochol, C., & Armony, J. L. (2008). The role of the amygdala in emotional processing: A quantitative meta-analysis of functional neuroimaging studies. *Neuroscience and Biobehavioral Reviews, 32*, 811–830. https://doi.org/10.1016/j.neubiorev.2007.12.002

Shah, A. K., Tioleco, N. M., Nolan, K., Locker, J., Groh, K., Villa, C., … Lachman, H. M. (2010). Rare NRXN1 promoter variants in patients with schizophrenia. *Neuroscience Letters, 475*(2), 80–84. https://doi.org/10.1016/J.NEULET.2010.03.047

Shah, P., Happé, F., Sowden, S., Cook, R., & Bird, G. (2015). Orienting Toward Face-Like Stimuli in Early Childhood. *Child Development, 86*(6), 1693–1700. https://doi.org/10.1111/cdev.12441

Shamay-Tsoory, S. G., Aharon-Peretz, J., & Perry, D. (2009). Two systems for empathy: a double dissociation between emotional and cognitive empathy in inferior frontal gyrus versus ventromedial prefrontal lesions. *Brain*, *132*(3), 617–627. https://doi.org/10.1093/brain/awn279

Shepherd, G. M. G., Pologruto, T. A., Svoboda, K., Wu, C. Z., Markram, H., & Luhmann, H. J. (2003). Circuit analysis of experience-dependent plasticity in the developing rat barrel cortex. *Neuron*, *38*(2), 277–89. https://doi.org/10.1016/S0896-6273(03)00152-1

Shibata, M., Fuchino, Y., Naoi, N., Kohno, S., Kawai, M., Okanoya, K., & Myowa-Yamakoshi, M. (2012). Broad cortical activation in response to tactile stimulation in newborns. *NeuroReport*, *23*(6), 373–377. https://doi.org/10.1097/WNR.0b013e3283520296

Shinohe, A., Hashimoto, K., Nakamura, K., Tsujii, M., Iwata, Y., Tsuchiya, K. J., … Mori, N. (2006). Increased serum levels of glutamate in adult patients with autism. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *30*(8), 1472–1477. https://doi.org/10.1016/J.PNPBP.2006.06.013

Silberberg, A., Allouch, C., Sandfort, S., Kearns, D., Karpel, H., & Slotnick, B. (2014). Desire for social contact, not empathy, may explain "rescue" behavior in rats. *Animal Cognition*, *17*(3), 609–618. https://doi.org/10.1007/s10071-013-0692-1

Silva-Gómez, A. B., Rojas, D., Juárez, I., & Flores, G. (2003). Decreased dendritic spine density on prefrontal cortical and hippocampal pyramidal neurons in postweaning social isolation rats. *Brain Research*, *983*(1–2), 128–136. https://doi.org/10.1016/S0006-8993(03)03042-7

Silva, B. A., Mattucci, C., Krzywkowski, P., Murana, E., Illarionova, A., Grinevich, V., …

Gross, C. T. (2013). Independent hypothalamic circuits for social and predator fear. *Nature Neuroscience*, *16*(12), 1731–3. https://doi.org/10.1038/nn.3573

Simion, F., Regolin, L., & Bulf, H. (2008). A predisposition for biological motion in the newborn baby. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(2), 809–13. https://doi.org/10.1073/pnas.0707021105

Singer, T., Seymour, B., O'Doherty, J., Kaube, H., Dolan, R. J., & Frith, C. D. (2004). Empathy for Pain Involves the Affective but not Sensory Components of Pain. *Science*, *303*(5661).

Slavotinek, A. M. (2008). Novel microdeletion syndromes detected by chromosome microarrays. *Human Genetics*, *124*(1), 1–17. https://doi.org/10.1007/s00439-008-0513-9

Soumiya, H., Godai, A., Araiso, H., Mori, S., Furukawa, S., & Fukumitsu, H. (2016). Neonatal Whisker Trimming Impairs Fear/Anxiety-Related Emotional Systems of the Amygdala and Social Behaviors in Adult Mice. *PloS One*, *11*(6), e0158583. https://doi.org/10.1371/journal.pone.0158583

Sowden, S., Koehne, S., Catmur, C., Dziobek, I., & Bird, G. (2016). Intact Automatic Imitation and Typical Spatial Compatibility in Autism Spectrum Disorder: Challenging the Broken Mirror Theory. *Autism Research*, *9*(2), 292–300. https://doi.org/10.1002/aur.1511

Sowell, E. R., Thompson, P. M., Holmes, C. J., Jernigan, T. L., & Toga, A. W. (1999). *In vivo* evidence for post-adolescent brain maturation in frontal and striatal regions. *Nature Neuroscience*, *2*(10), 859–861. https://doi.org/10.1038/13154

Stack, D. M., & Muir, D. W. (1992). Adult tactile stimulation during face-to-face interactions

modulates five-month-olds' affect and attention. *Child Development*, *63*(6), 1509–25.

Stefanacci, L., & Amaral, D. G. (2002). Some observations on cortical inputs to the macaque monkey amygdala: An anterograde tracing study. *The Journal of Comparative Neurology*, *451*(4), 301–323. https://doi.org/10.1002/cne.10339

Stefansson, H., Meyer-Lindenberg, A., Steinberg, S., Magnusdottir, B., Morgen, K., Arnarsdottir, S., … Stefansson, K. (2013). CNVs conferring risk of autism or schizophrenia affect cognition in controls. *Nature*, *505*(7483), 361–366. https://doi.org/10.1038/nature12818

Steinberg, L., & Morris, A. S. (2001). Adolescent Development. *Annual Review of Psychology*, *52*(1), 83–110. https://doi.org/10.1146/annurev.psych.52.1.83

Stern, E. A., Maravall, M., & Svoboda, K. (2001). Rapid Development and Plasticity of Layer 2/3 Maps in Rat Barrel Cortex In Vivo. *Neuron*, *31*(2), 305–315. https://doi.org/10.1016/S0896-6273(01)00360-9

Stevenson, R. J., & Miller, L. A. (2013). Taste and odour-induced taste perception following unilateral lesions to the anteromedial temporal lobe and the orbitofrontal cortex. *Cognitive Neuropsychology*, *30*(1), 41–57. https://doi.org/10.1080/02643294.2013.776526

Stone, V. E., Baron-Cohen, S., & Knight, R. T. (1998). Frontal Lobe Contributions to Theory of Mind. *Journal of Cognitive Neuroscience*, *10*(5), 640–656. https://doi.org/10.1162/089892998562942

Stoop, R. (2012). Neuromodulation by Oxytocin and Vasopressin. *Neuron*, *76*(1), 142–159. https://doi.org/10.1016/J.NEURON.2012.09.025

Strauss, K. A., Puffenberger, E. G., Huentelman, M. J., Gottlieb, S., Dobrin, S. E., Parod, J. M., … Morton, D. H. (2006). Recessive Symptomatic Focal Epilepsy and Mutant Contactin-Associated Protein-like 2. *New England Journal of Medicine*, *354*(13), 1370–1377. https://doi.org/10.1056/NEJMoa052773

Sugita, S., Khvochtev, M., & Südhof, T. C. (1999). Neurexins are functional alpha-latrotoxin receptors. *Neuron*, *22*(3), 489–96.

Sul, J. H., Kim, H., Huh, N., Lee, D., & Jung, M. W. (2010). Distinct roles of rodent orbitofrontal and medial prefrontal cortex in decision making. *Neuron*, *66*(3), 449–60. https://doi.org/10.1016/j.neuron.2010.03.033

Sullivan, P. F., Kendler, K. S., & Neale, M. C. (2003a). Schizophrenia as a Complex Trait. *Archives of General Psychiatry*, *60*(12), 1187. https://doi.org/10.1001/archpsyc.60.12.1187

Sullivan, R. M., Landers, M. S., Flemming, J., Vaught, C., Young, T. A., & Jonathan Polan, H. (2003). Characterizing the functional significance of the neonatal rat vibrissae prior to the onset of whisking. *Somatosensory & Motor Research*, *20*(2), 157–62. https://doi.org/10.1080/0899022031000105190

Sun, L., Min, L., Zhou, H., Li, M., Shao, F., & Wang, W. (2017). Adolescent social isolation affects schizophrenia-like behavior and astrocyte biomarkers in the PFC of adult rats. *Behavioural Brain Research*, *333*, 258–266. https://doi.org/10.1016/j.bbr.2017.07.011

Sundaram, S. K., Huq, A. M., Wilson, B. J., & Chugani, H. T. (2010). Tourette syndrome is associated with recurrent exonic copy number variants. *Neurology*, *74*(20), 1583–90. https://doi.org/10.1212/WNL.0b013e3181e0f147

Sungur, A. Ö., Schwarting, R. K. W., & Wöhr, M. (2017). Behavioral phenotypes and neurobiological mechanisms in the Shank1 mouse model for autism spectrum disorder: A translational perspective. *Behavioural Brain Research*. https://doi.org/10.1016/J.BBR.2017.09.038

Susilo, T., Germine, L., & Duchaine, B. (2013). Face recognition ability matures late: Evidence from individual differences in young adults. *Journal of Experimental Psychology: Human Perception and Performance*, *39*(5), 1212–1217. https://doi.org/10.1037/a0033469

Sztainberg, Y., & Zoghbi, H. Y. (2016). Lessons learned from studying syndromic autism spectrum disorders. *Nature Neuroscience*, *19*(11), 1408–1417. https://doi.org/10.1038/nn.4420

Tabuchi, K., Blundell, J., Etherton, M. R., Hammer, R. E., Liu, X., Powell, C. M., & Südhof, T. C. (2007). A neuroligin-3 mutation implicated in autism increases inhibitory synaptic transmission in mice. *Science (New York, N.Y.)*, *318*(5847), 71–6. https://doi.org/10.1126/science.1146221

Tager-Flusberg, H., Plesa-Skwerer, D., Faja, S., & Joseph, R. M. (2003). People with Williams syndrome process faces holistically. *Cognition*, *89*(1), 11–24. https://doi.org/10.1016/S0010-0277(03)00049-0

Tager-Flusberg, H., & Sullivan, K. (2000). A componential view of theory of mind: evidence from Williams syndrome. *Cognition*, *76*(1), 59–90. https://doi.org/10.1016/S0010-0277(00)00069-X

Takahashi, A., Nagayasu, K., Nishitani, N., Kaneko, S., & Koide, T. (2014). Control of Intermale Aggression by Medial Prefrontal Cortex Activation in the Mouse. *PLoS ONE*,

9(4), e94657. https://doi.org/10.1371/journal.pone.0094657

Takayanagi, Y., Takahashi, T., Orikabe, L., Masuda, N., Mozue, Y., Nakamura, K., … Suzuki, M. (2010). Volume reduction and altered sulco-gyral pattern of the orbitofrontal cortex in first-episode schizophrenia. *Schizophrenia Research*, *121*(1–3), 55–65. https://doi.org/10.1016/j.schres.2010.05.006

Takayanagi, Y., Yoshida, M., Bielsky, I. F., Ross, H. E., Kawamata, M., Onaka, T., … Nishimori, K. (2005). Pervasive social deficits, but normal parturition, in oxytocin receptor-deficient mice. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(44), 16096–101. https://doi.org/10.1073/pnas.0505312102

Takita, M., & Kikusui, T. (2016). Early weaning influences short-term synaptic plasticity in the medial prefrontal–anterior basolateral amygdala pathway. *Neuroscience Research*, *103*, 48–53. https://doi.org/10.1016/J.NEURES.2015.08.003

Tanabe, T., Iino, M., & Takagi, S. F. (1975). Discrimination of odors in olfactory bulb, pyriform-amygdaloid areas, and orbitofrontal cortex of the monkey. *Journal of Neurophysiology*, *38*(5), 1284–96.

Thierry, A. M., Gioanni, Y., Dégénétais, E., & Glowinski, J. (2000). Hippocampo-prefrontal cortex pathway: anatomical and electrophysiological characteristics. *Hippocampus*, 10(4), 411-419.

Thomas, M. S. C., Davis, R., Karmiloff-Smith, A., Knowland, V. C. P., & Charman, T. (2016). The over-pruning hypothesis of autism. *Developmental Science*, *19*(2), 284–305. https://doi.org/10.1111/desc.12303

Thompson, J. C., Clarke, M., Stewart, T., & Puce, A. (2005). Configural processing of

biological motion in human superior temporal sulcus. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *25*(39), 9059–66. https://doi.org/10.1523/JNEUROSCI.2129-05.2005

Thompson, R. R., George, K., Walton, J. C., Orr, S. P., & Benson, J. (2006). Sex-specific influences of vasopressin on human social communication. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(20), 7889–94. https://doi.org/10.1073/pnas.0600406103

Thomson, A. M. (1997). Activity-dependent properties of synaptic transmission at two classes of connections made by rat neocortical pyramidal axons *in vitro*. *The Journal of Physiology*, *502*(1), 131–147. https://doi.org/10.1111/j.1469-7793.1997.131bl.x

Thor, D. H., & Holloway, W. R. (1984). Developmental analyses of social play behavior in juvenile rats. *Bulletin of the Psychonomic Society*, *22*(6), 587–590. https://doi.org/10.3758/BF03333916

Timbie, C., & Barbas, H. (2014). Specialized pathways from the primate amygdala to posterior orbitofrontal cortex. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *34*(24), 8106–18. https://doi.org/10.1523/JNEUROSCI.5014-13.2014

Todarello, G., Feng, N., Kolachana, B. S., Li, C., Vakkalanka, R., Bertolino, A., … Straub, R. E. (2014). Incomplete penetrance of NRXN1 deletions in families with schizophrenia. *Schizophrenia Research*, *155*(1–3), 1–7. https://doi.org/10.1016/j.schres.2014.02.023

Tomaiuolo, F., Di Paola, M., Caravale, B., Vicari, S., Petrides, M., & Caltagirone, C. (2002). Morphology and morphometry of the corpus callosum in Williams syndrome: a T1-weighted MRI study. *Neuroreport*, *13*(17), 2281–4.

https://doi.org/10.1097/01.wnr.0000044222.79663.72

Tomalski, P., Csibra, G., & Johnson, M. H. (2009). Rapid Orienting toward Face-like Stimuli with Gaze-Relevant Contrast Information. *Perception*, *38*(4), 569–578. https://doi.org/10.1068/p6137

Tomasello, M., Carpenter, M., & Liszkowski, U. (2007). A New Look at Infant Pointing. *Child Development*, *78*(3), 705–722. https://doi.org/10.1111/j.1467-8624.2007.01025.x

Tomchek, S. D., & Dunn, W. (2007). Sensory Processing in Children With and Without Autism: A Comparative Study Using the Short Sensory Profile. *American Journal of Occupational Therapy*, *61*(2), 190–200. https://doi.org/10.5014/ajot.61.2.190

Tremblay, L., & Schultz, W. (2000). Reward-related neuronal activity during go-nogo task performance in primate orbitofrontal cortex. *Journal of Neurophysiology*, *83*(4), 1864–76.

Trezza, V., Baarendse, P. J. J., & Vanderschuren, L. J. M. J. (2010). The pleasures of play: pharmacological insights into social reward mechanisms. *Trends in Pharmacological Sciences*, *31*(10), 463–469. https://doi.org/10.1016/J.TIPS.2010.06.008

Turecek, J., Jackman, S. L., & Regehr, W. G. (2017). Synaptotagmin 7 confers frequency invariance onto specialized depressing synapses. *Nature*, *551*(7681). https://doi.org/10.1038/nature24474

Twining, R. C., Vantrease, J. E., Love, S., Padival, M., & Rosenkranz, J. A. (2017). An intra-amygdala circuit specifically regulates social fear learning. *Nature Neuroscience*, *20*(3), 459–469. https://doi.org/10.1038/nn.4481

Uchiyama, H., Seki, A., Kageyama, H., Saito, D. N., Koeda, T., Ohno, K., & Sadato, N.

(2006). Neural substrates of sarcasm: A functional magnetic-resonance imaging study. *Brain Research*, *1124*(1), 100–110. https://doi.org/10.1016/J.BRAINRES.2006.09.088

Udwin, O., & Yule, W. (1991). A cognitive and behavioural phenotype in williams syndrome. *Journal of Clinical and Experimental Neuropsychology*, *13*(2), 232–244. https://doi.org/10.1080/01688639108401040

Ueno, H., Shoshi, C., Suemitsu, S., Usui, S., Sujiura, H., & Okamoto, M. (2013). Somatosensory and visual deprivation each decrease the density of parvalbumin neurons and their synapse terminals in the prefrontal cortex and hippocampus of mice. *Acta Medica Okayama*, *67*(3), 135–43. https://doi.org/10.18926/AMO/50406

Ullrich, B., Ushkaryov, Y. A., & Südhof, T. C. (1995). Cartography of neurexins: More than 1000 isoforms generated by alternative splicing and expressed in distinct subsets of neurons. *Neuron*, *14*(3), 497–507. https://doi.org/10.1016/0896-6273(95)90306-2

Ushkaryov, Y. A., Petrenko, A. G., Geppert, M., & Südhof, T. C. (1992). Neurexins: synaptic cell surface proteins related to the alpha-latrotoxin receptor and laminin. *Science (New York, N.Y.)*, *257*(5066), 50–6.

Uylings, H. B. M., Groenewegen, H. J., & Kolb, B. (2003). Do rats have a prefrontal cortex? *Behavioural Brain Research*, *146*(1–2), 3–17. https://doi.org/10.1016/J.BBR.2003.09.028

Vaags, A. K., Lionel, A. C., Sato, D., Goodenberger, M., Stein, Q. P., Curran, S., … Scherer, S. W. (2012). Rare Deletions at the Neurexin 3 Locus in Autism Spectrum Disorder. *The American Journal of Human Genetics*, *90*(1), 133–141. https://doi.org/10.1016/J.AJHG.2011.11.025

Vaish, A., Carpenter, M., & Tomasello, M. (2010). Young Children Selectively Avoid Helping People With Harmful Intentions. *Child Development*, *81*(6), 1661–1669. https://doi.org/10.1111/j.1467-8624.2010.01500.x

Vaish, A., & Striano, T. (2004). Is visual reference necessary? Contributions of facial versus vocal cues in 12-month-olds' social referencing behavior. *Developmental Science*, *7*(3), 261–269. https://doi.org/10.1111/j.1467-7687.2004.00344.x

Valenza, E., Simion, F., Cassia, V. M., & Umiltà, C. (1996). Face preference at birth. *Journal of Experimental Psychology: Human Perception and Performance*, *22*(4), 892–903. https://doi.org/10.1037/0096-1523.22.4.892

van den Berg, C. L., Hol, T., Van Ree, J. M., Spruijt, B. M., Everts, H., & Koolhaas, J. M. (1999). Play is indispensable for an adequate development of coping with social challenges in the rat. *Developmental Psychobiology*, *34*(2), 129–138. https://doi.org/10.1002/(SICI)1098-2302(199903)34:2<129::AID-DEV6>3.0.CO;2-L

van Gaalen, M. M., & Steckler, T. (2000). Behavioural analysis of four mouse strains in an anxiety test battery. *Behavioural Brain Research*, *115*(1), 95–106. https://doi.org/10.1016/S0166-4328(00)00240-0

van Leengoed, E., Kerker, E., & Swanson, H. H. (1987). Inhibition of post-partum maternal behaviour in the rat by injecting an oxytocin antagonist into the cerebral ventricles. *The Journal of Endocrinology*, *112*(2), 275–82. https://doi.org/10.1677/JOE.0.1120275

Vanderschuren, L. J. M. J., Achterberg, E. J. M., & Trezza, V. (2016). The neurobiology of social play and its rewarding value in rats. *Neuroscience & Biobehavioral Reviews*, *70*, 86–105. https://doi.org/10.1016/j.neubiorev.2016.07.025

Vassos, E., Collier, D. A., Holden, S., Patch, C., Rujescu, D., St Clair, D., & Lewis, C. M. (2010). Penetrance for copy number variants associated with schizophrenia. *Human Molecular Genetics*, *19*(17), 3477–3481. https://doi.org/10.1093/hmg/ddq259

Vecera, S. P., & Johnson, M. H. (1995). Gaze detection and the cortical processing of faces: Evidence from infants and adults. *Visual Cognition*, *2*(1), 59–87. https://doi.org/10.1080/13506289508401722

Verkerk, A. J. M. H., Pieretti, M., Sutcliffe, J. S., Fu, Y.-H., Kuhl, D. P. A., Pizzuti, A., … Warren, S. T. (1991). Identification of a gene (FMR-1) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. *Cell*, *65*(5), 905–914. https://doi.org/10.1016/0092-8674(91)90397-H

Villalobos, M. E., Mizuno, A., Dahl, B. C., Kemmotsu, N., & Müller, R.-A. (2005). Reduced functional connectivity between V1 and inferior frontal cortex associated with visuomotor performance in autism. *NeuroImage*, *25*(3), 916–925. https://doi.org/10.1016/J.NEUROIMAGE.2004.12.022

Viñas-Jornet, M., Esteba-Castillo, S., Gabau, E., Ribas-Vidal, N., Baena, N., San, J., … Guitart, M. (2014). A common cognitive, psychiatric, and dysmorphic phenotype in carriers of *NRXN1* deletion. *Molecular Genetics & Genomic Medicine*, *2*(6), 512–521. https://doi.org/10.1002/mgg3.105

Viskontas, I. V., Possin, K. L., & Miller, B. L. (2007). Symptoms of Frontotemporal Dementia Provide Insights into Orbitofrontal Cortex Function and Social Behavior. *Annals of the New York Academy of Sciences*, *1121*(1), 528–545. https://doi.org/10.1196/annals.1401.025

Völlm, B. A., Taylor, A. N. W., Richardson, P., Corcoran, R., Stirling, J., McKie, S., …

Elliott, R. (2006). Neuronal correlates of theory of mind and empathy: A functional magnetic resonance imaging study in a nonverbal task. *NeuroImage*, *29*(1), 90–98. https://doi.org/10.1016/J.NEUROIMAGE.2005.07.022

Von Der Heide, R., Vyas, G., & Olson, I. R. (2014). The social network-network: size is predicted by brain structure and function in the amygdala and paralimbic regions. *Social Cognitive and Affective Neuroscience*, *9*(12), 1962–1972. https://doi.org/10.1093/scan/nsu009

Voos, A. C., Pelphrey, K. A., & Kaiser, M. D. (2013). Autistic traits are associated with diminished neural response to affective touch. *Social Cognitive and Affective Neuroscience*, *8*(4), 378–386. https://doi.org/10.1093/scan/nss009

Walden, T. A., & Ogan, T. A. (1988). The development of social referencing. *Child Development*, *59*(5), 1230–40.

Walker, A. E. (1940). A cytoarchitectural study of the prefrontal area of the macaque monkey. *The Journal of Comparative Neurology*, *73*(1), 59–86. https://doi.org/10.1002/cne.900730106

Walker, E., Marwit, S., & Emory, E. (1980). A cross-sectional study of emotion recognition in schizophrenics. *Journal of Abnormal Psychology*, *89*(3), 428–436. https://doi.org/10.1037/0021-843X.89.3.428

Wallace, B., Cesarini, D., Lichtenstein, P., & Johannesson, M. (2007). Heritability of ultimatum game responder behavior. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(40), 15631–4. https://doi.org/10.1073/pnas.0706642104

Walle, E. A., & Campos, J. J. (2014). The development of infant detection of inauthentic emotion. *Emotion (Washington, D.C.), 14*(3), 488–503. https://doi.org/10.1037/a0035305

Wallis, J. D. (2007). Orbitofrontal Cortex and Its Contribution to Decision-Making. *Annual Review of Neuroscience*, *30*(1), 31–56. https://doi.org/10.1146/annurev.neuro.30.051606.094334

Walton, G. E., Bower, N. J. A., & Bower, T. G. R. (1992). Recognition of familiar faces by newborns. *Infant Behavior and Development*, *15*(2), 265–269. https://doi.org/10.1016/0163-6383(92)80027-R

Walum, H., Westberg, L., Henningsson, S., Neiderhiser, J. M., Reiss, D., Igl, W., … Lichtenstein, P. (2008). Genetic variation in the vasopressin receptor 1a gene (AVPR1A) associates with pair-bonding behavior in humans. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(37), 14153–6. https://doi.org/10.1073/pnas.0803081105

Wang, X.-J. (1999). Fast burst firing and short-term synaptic plasticity: A model of neocortical chattering neurons. *Neuroscience*, *89*(2), 347–362. https://doi.org/10.1016/S0306-4522(98)00315-7

Wang, X.-J. (2001). Synaptic reverberation underlying mnemonic persistent activity. *Trends in Neurosciences*, *24*(8), 455–463. https://doi.org/10.1016/S0166-2236(00)01868-3

Wang, Y., Markram, H., Goodman, P. H., Berger, T. K., Ma, J., & Goldman-Rakic, P. S. (2006). Heterogeneity in the pyramidal network of the medial prefrontal cortex. *Nature Neuroscience*, *9*(4), 534–542. https://doi.org/10.1038/nn1670

Warneken, F., Gräfenhain, M., & Tomasello, M. (2012). Collaborative partner or social tool? New evidence for young children's understanding of joint intentions in collaborative activities. *Developmental Science*, *15*(1), 54–61. https://doi.org/10.1111/j.1467-7687.2011.01107.x

Warneken, F., & Tomasello, M. (2007). Helping and Cooperation at 14 Months of Age. *Infancy*, *11*(3), 271–294. https://doi.org/10.1111/j.1532-7078.2007.tb00227.x

Watanabe, H., Nakamura, M., Ohno, T., Itahashi, T., Tanaka, E., Ohta, H., … Hashimoto, R. (2014). Altered orbitofrontal sulcogyral patterns in adult males with high-functioning autism spectrum disorders. *Social Cognitive and Affective Neuroscience*, *9*(4), 520–528. https://doi.org/10.1093/scan/nst016

Watson, L. R., Patten, E., Baranek, G. T., Poe, M., Boyd, B. A., Freuler, A., & Lorenzi, J. (2011). Differential Associations Between Sensory Response Patterns and Language, Social, and Communication Measures in Children With Autism or Other Developmental Disabilities. *Journal of Speech Language and Hearing Research*, *54*(6), 1562. https://doi.org/10.1044/1092-4388(2011/10-0029)

Welchew, D. E., Ashwin, C., Berkouk, K., Salvador, R., Suckling, J., Baron-Cohen, S., & Bullmore, E. (2005). Functional disconnectivity of the medial temporal lobe in Asperger's syndrome. *Biological Psychiatry*, *57*(9), 991–998. https://doi.org/10.1016/J.BIOPSYCH.2005.01.028

Welker, W. I. (1964). Analysis of Sniffing of the Albino Rat. *Behaviour*. Brill. https://doi.org/10.2307/4533073

Wen, J. A., & Barth, A. L. (2011). Input-Specific Critical Periods for Experience-Dependent Plasticity in Layer 2/3 Pyramidal Neurons. *Journal of Neuroscience*, *31*(12). Retrieved

from http://www.jneurosci.org/content/31/12/4456.long

Weng, S.-M., McLeod, F., Bailey, M. E. S., & Cobb, S. R. (2011). Synaptic plasticity deficits in an experimental model of rett syndrome: long-term potentiation saturation and its pharmacological reversal. *Neuroscience*, *180*, 314–321. https://doi.org/10.1016/J.NEUROSCIENCE.2011.01.061

Wiggins, L. D., Robins, D. L., Bakeman, R., & Adamson, L. B. (2009). Breif Report: Sensory Abnormalities as Distinguishing Symptoms of Autism Spectrum Disorders in Young Children. *Journal of Autism and Developmental Disorders*, *39*(7), 1087–1091. https://doi.org/10.1007/s10803-009-0711-x

Wilhelm, B. G., Groemer, T. W., & Rizzoli, S. O. (2010). The same synaptic vesicles drive active and spontaneous release. *Nature neuroscience*, 13(12), 1454-1456.

Willey, A. R., & Spear, L. P. (2012). Development of anticipatory 50 kHz USV production to a social stimuli in adolescent and adult male Sprague-Dawley rats. *Behavioural Brain Research*, *226*(2), 613–618. https://doi.org/10.1016/J.BBR.2011.10.001

Willis, M. L., Murphy, J. M., Ridley, N. J., & Vercammen, A. (2015). Anodal tDCS targeting the right orbitofrontal cortex enhances facial expression recognition. *Social Cognitive and Affective Neuroscience*, *10*(12), 1677–1683. https://doi.org/10.1093/scan/nsv057

Willis, M. L., Palermo, R., Burke, D., McGrillen, K., & Miller, L. (2010). Orbitofrontal cortex lesions result in abnormal social judgements to emotional faces. *Neuropsychologia*, *48*(7), 2182–2187. https://doi.org/10.1016/J.NEUROPSYCHOLOGIA.2010.04.010

Wilson, R. C., Takahashi, Y. K., Schoenbaum, G., & Niv, Y. (2014). Orbitofrontal Cortex as

a Cognitive Map of Task Space. *Neuron*, *81*(2), 267–279. https://doi.org/10.1016/J.NEURON.2013.11.005

Wimmer, H. (1983). Beliefs about beliefs: Representation and constraining function of wrong beliefs in young children's understanding of deception. *Cognition*, *13*(1), 103–128. https://doi.org/10.1016/0010-0277(83)90004-5

Wing, L., & Gould, J. (1979). Severe Impairments of Social Interaction and Associated Abnormalities in Children: Epidemiology and Classification. *Journal of Autism and Developmental Disorders*, *9*(1).

Winslow, J. T., Hastings, N., Carter, C. S., Harbaugh, C. R., & Insel, T. R. (1993). A role for central vasopressin in pair bonding in monogamous prairie voles. *Nature*, *365*(6446), 545–548. https://doi.org/10.1038/365545a0

Winslow, J. T., Hearn, E. F., Ferguson, J., Young, L. J., Matzuk, M. M., & Insel, T. R. (2000). Infant Vocalization, Adult Aggression, and Fear Behavior of an Oxytocin Null Mutant Mouse. *Hormones and Behavior*, *37*(2), 145–155. https://doi.org/10.1006/HBEH.1999.1566

Winslow, J. T., & Insel, T. R. (2002). The social deficits of the oxytocin knockout mouse. *Neuropeptides*, *36*(2–3), 221–229. https://doi.org/10.1054/NPEP.2002.0909

Wöhr, M., & Scattoni, M. L. (2013). Behavioural methods used in rodent models of autism spectrum disorders: Current standards and new developments. *Behavioural Brain Research*, *251*, 5–17. https://doi.org/10.1016/j.bbr.2013.05.047

Wöhr, M., Schwarting, R. K. W., Magnuson, T., Crawley, J., & Wilson, E. (2007). Ultrasonic Communication in Rats: Can Playback of 50-kHz Calls Induce Approach Behavior?

PLoS ONE, *2*(12), e1365. https://doi.org/10.1371/journal.pone.0001365

Wolfe, J., Mende, C., & Brecht, M. (2011). Social facial touch in rats. *Behavioral Neuroscience*, *125*(6), 900–910. https://doi.org/10.1037/a0026165

Won, H., Lee, H.-R., Gee, H. Y., Mah, W., Kim, J.-I., Lee, J., … Kim, E. (2012). Autistic-like social behaviour in Shank2-mutant mice improved by restoring NMDA receptor function. *Nature*, *486*(7402), 261–265. https://doi.org/10.1038/nature11208

Wong, L. C., Wang, L., D'Amour, J. A., Yumita, T., Chen, G., Yamaguchi, T., … Lin, D. (2016). Effective Modulation of Male Aggression through Lateral Septum to Medial Hypothalamus Projection. *Current Biology*, *26*(5), 593–604. https://doi.org/10.1016/J.CUB.2015.12.065

Wood, L., Gray, N. W., Zhou, Z., Greenberg, M. E., & Shepherd, G. M. G. (2009). Synaptic Circuit Abnormalities of Motor-Frontal Layer 2/3 Pyramidal Neurons in an RNA Interference Model of Methyl-CpG-Binding Protein 2 Deficiency. *Journal of Neuroscience*, *29*(40), 12440–12448. https://doi.org/10.1523/JNEUROSCI.3321-09.2009

Woolsey, T. A., & Van der Loos, H. (1970). The structural organization of layer IV in the somatosensory region (SI) of mouse cerebral cortex: the description of a cortical field composed of discrete cytoarchitectonic units. Brain research, 17(2), 205-242.

Woolsey, T. A., & Wann, J. R. (1976). Areal changes in mouse cortical barrels following vibrissal damage at different postnatal ages. *The Journal of Comparative Neurology*, *170*(1), 53–66. https://doi.org/10.1002/cne.901700105

Wray, N. R., Goddard, M. E., & Visscher, P. M. (2008). Prediction of individual genetic risk

of complex disease. *Current Opinion in Genetics & Development*, *18*(3), 257–263. https://doi.org/10.1016/j.gde.2008.07.006

Wu, H.-P. P., Ioffe, J. C., Iverson, M. M., Boon, J. M., & Dyck, R. H. (2013). Novel, whisker-dependent texture discrimination task for mice. *Behavioural Brain Research*, *237*, 238–242. https://doi.org/10.1016/J.BBR.2012.09.044

Xu, B., Roos, J. L., Levy, S., van Rensburg, E. J., Gogos, J. A., & Karayiorgou, M. (2008). Strong association of de novo copy number mutations with sporadic schizophrenia. *Nature Genetics*, *40*(7), 880–885. https://doi.org/10.1038/ng.162

Xu, J., Pang, Z. P., Shin, O.-H., & Südhof, T. C. (2009). Synaptotagmin-1 functions as a Ca2+ sensor for spontaneous release. *Nature Neuroscience*, *12*(6), 759–766. https://doi.org/10.1038/nn.2320

Yan, J., Noltner, K., Feng, J., Li, W., Schroer, R., Skinner, C., … Sommer, S. S. (2008). Neurexin 1α structural variants associated with autism. *Neuroscience Letters*, *438*(3), 368–370. https://doi.org/10.1016/J.NEULET.2008.04.074

Yang, M., & Crawley, J. N. (2009). Simple behavioral assessment of mouse olfaction. *Current protocols in neuroscience*, 8-24.

Yang, M., Silverman, J. L., & Crawley, J. N. (2011). Automated three-chambered social approach task for mice. *Current protocols in neuroscience*, 8-26.

Yangngam, S., Plong-On, O., Sripo, T., Roongpraiwan, R., Hansakunachai, T., Wirojanan, J., … Limprasert, P. (2014). Mutation Screening of the Neurexin 1 Gene in Thai Patients with Intellectual Disability and Autism Spectrum Disorder. *Genetic Testing and Molecular Biomarkers*, *18*(7), 510–515. https://doi.org/10.1089/gtmb.2014.0003

Yeung, N., Botvinick, M. M., & Cohen, J. D. (2004). The Neural Basis of Error Detection: Conflict Monitoring and the Error-Related Negativity. *Psychological Review*, *111*(4), 931–959. https://doi.org/10.1037/0033-295X.111.4.931

Yizhar, O., Fenno, L. E., Prigge, M., Schneider, F., Davidson, T. J., O'Shea, D. J., … Deisseroth, K. (2011a). Neocortical excitation/inhibition balance in information processing and social dysfunction. *Nature*, *477*(7363), 171–178. https://doi.org/10.1038/nature10360

Yizhar, O., Fenno, L. E., Prigge, M., Schneider, F., Davidson, T. J., O'Shea, D. J., … Deisseroth, K. (2011b). Neocortical excitation/inhibition balance in information processing and social dysfunction. *Nature*, *477*(7363), 171–8. https://doi.org/10.1038/nature10360

Young, L., Camprodon, J. A., Hauser, M., Pascual-Leone, A., & Saxe, R. (2010). Disruption of the right temporoparietal junction with transcranial magnetic stimulation reduces the role of beliefs in moral judgments. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(15), 6753–8. https://doi.org/10.1073/pnas.0914826107

Zahn, R., Green, S., Beaumont, H., Burns, A., Moll, J., Caine, D., … Lambon Ralph, M. A. (2017a). Frontotemporal lobar degeneration and social behaviour: Dissociation between the knowledge of its consequences and its conceptual meaning. *Cortex*, *93*, 107–118. https://doi.org/10.1016/j.cortex.2017.05.009

Zahn, R., Green, S., Beaumont, H., Burns, A., Moll, J., Caine, D., … Lambon Ralph, M. A. (2017b). Frontotemporal lobar degeneration and social behaviour: Dissociation between the knowledge of its consequences and its conceptual meaning. *Cortex*, *93*, 107–118.

https://doi.org/10.1016/j.cortex.2017.05.009

Zahn, R., Moll, J., Iyengar, V., Huey, E. D., Tierney, M., Krueger, F., & Grafman, J. (2009). Social conceptual impairments in frontotemporal lobar degeneration with right anterior temporal hypometabolism. *Brain*, *132*(3), 604–616. https://doi.org/10.1093/brain/awn343

Zahn, R., Moll, J., Krueger, F., Huey, E. D., Garrido, G., & Grafman, J. (2007). Social concepts are represented in the superior anterior temporal cortex. *Proceedings of the National Academy of Sciences*, *104*(15), 6430–6435. https://doi.org/10.1073/pnas.0607061104

Zahn, R., Moll, J., Paiva, M., Garrido, G., Krueger, F., Huey, E. D., & Grafman, J. (2009). The Neural Basis of Human Social Values: Evidence from Functional MRI. *Cerebral Cortex*, *19*(2), 276–283. https://doi.org/10.1093/cercor/bhn080

Zatorre, R. J., Fields, R. D., & Johansen-Berg, H. (2012). Plasticity in gray and white: neuroimaging changes in brain structure during learning. *Nature Neuroscience*, *15*(4), 528–36. https://doi.org/10.1038/nn.3045

Zhan, Y., Paolicelli, R. C., Sforazzini, F., Weinhard, L., Bolasco, G., Pagani, F., … Gross, C. T. (2014). Deficient neuron-microglia signaling results in impaired functional brain connectivity and social behavior. *Nature Neuroscience*, *17*(3), 400–6. https://doi.org/10.1038/nn.3641

Zhang, Y., Zu, X., Luo, W., Yang, H., Luo, G., Zhang, M., & Tang, S. (2012). Social isolation produces anxiety-like behaviors and changes PSD-95 levels in the forebrain. *Neuroscience Letters*, *514*(1), 27–30. https://doi.org/10.1016/j.neulet.2012.02.043

Zhang, Z. W. (2004). Maturation of layer V pyramidal neurons in the rat prefrontal cortex: intrinsic properties and synaptic function. Journal of neurophysiology, 91(3), 1171-1182.

Zimmerman, A., Bai, L., & Ginty, D. D. (2014). The gentle touch receptors of mammalian skin. *Science (New York, N.Y.)*, *346*(6212), 950–4. https://doi.org/10.1126/science.1254229

Zucker, R. S. (1989). Short-term synaptic plasticity. *Ann. Rev. Neurosci*, *12*, 13–31.

Zwaigenbaum, L., Bryson, S., Rogers, T., Roberts, W., Brian, J., & Szatmari, P. (2005). Behavioral manifestations of autism in the first year of life. *International Journal of Developmental Neuroscience*, *23*(2–3), 143–152. https://doi.org/10.1016/J.IJDEVNEU.2004.05.001

Zweier, C., de Jong, E. K., Zweier, M., Orrico, A., Ousager, L. B., Collins, A. L., … Rauch, A. (2009). CNTNAP2 and NRXN1 Are Mutated in Autosomal-Recessive Pitt-Hopkins-like Mental Retardation and Determine the Level of a Common Synaptic Protein in Drosophila. *The American Journal of Human Genetics*, *85*(5), 655–666. https://doi.org/10.1016/J.AJHG.2009.10.004

Appendices

Appendix 1

Tables of means ± sem for behavioural tasks in Chapter 2.

6.5.1 Open Field

Table 8: Mean ± sem distance explored in open field

Group	Male	Female
WT	6445 ± 322 cm	6300 ± 290 cm
HET	6202 ± 228 cm	6402 ± 266 cm
KO	5415 ± 18 cm	5540 ± 28 cm

Table 9: Mean ± sem time in centre of open field

Group	Male	Female
WT	74.60 ± 4.90 s	89.69 ± 6.10 s
HET	82.70 ± 6.65 s	88.98 ± 5.31 s
KO	63.80 ± 5.58 s	50.13 ± 4.12 s

Table 10: Mean ± sem number of entries into centre of open field

Group	Male	Female
WT	75.64 ± 4.61	79 ± 3.64
HET	79.90 ± 3.31	76.79 ± 4.71
KO	54.78 ± 3.39	46.21 ± 2.92

6.5.2 3-Chamber Social Approach

Table 11: Mean ± sem distance covered in trial 1 of 3-chamber

Group	**Distance covered (cm)**
WT M	4928 ± 119 cm
WT F	5219 ± 137 cm
HET M	4991 ± 103 cm
HET F	5150 ± 175 cm
KO M	4311 ± 85 cm
KO F	4372 ± 171 cm

Table 12: Mean ± sem time in each chamber in trial 2 of 3-chamber

Group	Social Chamber	Middle Chamber	Non-social Chamber
WT M	259 ± 19 s	15 ± 3 s	164 ± 9 s
WT F	310 ± 14 s	22 ± 2 s	128 ± 12 s
HET M	271 ± 18 s	23 ± 3 s	157 ± 8 s
HET F	322 ± 14 s	25 ± 2 s	134 ± 9 s
KO M	346 ± 16 s	28 ± 3 s	126 ± 7 s
KO F	361 ± 23 s	26 ± 3 s	130 ± 13 s

Table 13: Mean ± sem time in each chamber in trial 3 of 3-chamber

Group	Familiar Chamber	Middle Chamber	Novel Chamber
WT M	186 ± 16 s	10 ± 3 s	256 ± 16 s
WT F	132 ± 10 s	29 ± 4 s	258 ± 22 s
HET M	210 ± 13 s	15 ± 2 s	217 ± 10 s
HET F	151 ± 13 s	30 ± 4 s	250 ± 17 s
KO M	224 ± 14 s	28 ± 3 s	233 ± 11 s
KO F	147 ± 18 s	38 ± 7 s	301 ± 18 s

6.5.3 Novel Object Discrimination

Table 14: Mean ± sem object exploration in trial 1 of novel object task

Group	Time investigating objects (s)	Number of object investigations
WT M	84 ± 5 s	92 ± 2
WT F	84 ± 8 s	96 ± 6
HET M	101 ± 6 s	98 ± 3
HET F	92 ± 4 s	108 ± 4
KO M	78 ± 3 s	85 ± 3
KO F	73 ± 5 s	97 ± 4

Table 15: Mean ± sem novelty discrimination in trial 2 of novel object task

Group	% novelty preference at 2 mins	% novelty preference at 5 mins	% novelty preference at 10 mins
WT M	61 ± 4 %	55 ± 2 %	57 ± 1 %
WT F	57 ± 3 %	53 ± 4 %	48 ± 2 %
HET M	61 ± 4 %	52 ± 2 %	51 ± 2 %
HET F	52 ± 3 %	51 ± 3 %	50 ± 2 %
KO M	54 ± 4 %	51 ± 2 %	49 ± 4 %
KO F	59 ± 6 %	57 ± 4 %	52 ± 4 %

6.5.4 Spontaneous Y Maze Spatial Novelty Discrimination

Table 16: Mean ± sem novelty preference in trial 2 of spontaneous Y maze spatial novelty discrimination task

Group	% novelty preference
WT M	64 ± 3 %
WT F	57 ± 7 %
HET M	62 ± 3 %
HET F	68 ± 1 %
KO M	63 ± 5 %
KO F	58 ± 6 %

6.5.5 Morris Water Maze

Spatial learning

Table 17: Mean ± sem path length in Morris water maze

Group	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
WT M	417 ± 70 cm	238 ± 41 cm	183 ± 35 cm	351 ± 57 cm	294 ± 51 cm	270 ± 52	149 ± 21 cm
WT F	494 ± 102 cm	244 ± 34 cm	264 ± 52 cm	195 ± 38 cm	181 ± 19 cm	193 ± 32 cm	186 ± 25 cm
HET M	360 ± 62 cm	258 ± 40 cm	239 ± 23 cm	325 ± 84 cm	235 ± 41 cm	195 ± 56 cm	109 ± 11 cm
HET F	313 ± 57 cm	214 ± 46 cm	199 ± 40 cm	197 ± 35 cm	117 ± 36 cm	201 ± 33 cm	182 ± 47 cm
KO M	391 ±85 cm	221 ±37 cm	304 ±56 cm	280 ± 54 cm	282 ±62 cm	198. ± 37 cm	139. ±19 cm
KO F	288 ± 52 cm	266 ± 57 cm	296 ± 43 cm	178 ± 25 cm	120 ± 19 cm	152 ± 20 cm	158 ± 35 cm

Table 18: Mean ± sem time (s) in quadrant during probe trial in Morris water maze

Group	Left	Opposite	Right	Target
WT M	13 ± 1 s	11 ± 2 s	12 ± 2 s	18 ± 2 s
WT F	13 ± 1	12 ± 1 s	13 ± 1 s	17 ± 2
HET M	12 ± 1 s	10 ± 1 s	12 ± 1 s	21 ± 2
HET F	10 ± 2 s	12 ± 3 s	14 ± 2 s	20 ± 2 s
KO M	12 ± 2 s	9 ± 2 s	12 ± 1 s	21 ± 1 s
KO F	14 ± 2 s	9 ± 2 s	12 ± 5 s	24 ± 3 s

Reversal Learning

Table 19: Mean ± sem path length in reversed Morris water maze

Group	Day 1	Day 2	Day 3	Day 4	Day 5
WT M	411 ± 89 cm	358 ± 83 cm	350 ± 66 cm	194 ± 39 cm	247 ± 91 cm
WT F	405 ± 69 cm	385 ± 52 cm	324 ± 48 cm	208 ± 43 cm	288 ± 49 cm
HET M	639 ± 89 cm	402 ± 39 cm	236 ± 40 cm	238 ± 54 cm	174 ± 40 cm
HET F	487 ± 103 cm	504 ± 135 cm	278 ± 53 cm	211 ± 15 cm	152 ± 25 cm
KO M	558 ± 80 cm	450 ± 56 cm	323 ± 101 cm	251 ± 52 cm	171 ± 24 cm
KO F	504 ± 75 cm	314 ± 29 cm	307 ± 49 cm	215 ± 45 cm	164 ± 25 cm

Table 20: Mean ± sem time in quadrant in probe trial in reversed Morris water maze

Group	Left	Opposite	Right	Target
WT M	10 ± 1 s	20 ± 2 s	15 ± 8 s	9 ± 1 s
WT F	11 ± 1 s	18 ± 2 s	19 ± 2 s	9 ± 1 s
HET M	11 ± 2 s	17 ± 1 s	15 ± 1 s	9 ± 1 s
HET F	11 ± 2 s	23 ± 2 s	13 ± 2 s	9 ± 2 s
KO M	10 ± 1 s	22 ± 2 s	16 ± 2 s	8 ± 2 s
KO F	10 ± 1 s	20 ± 1 s	16 ± 2 s	10 ± 1 s

Puzzle Box

Table 21: Mean latency to "goal" box on day 1 of puzzle box

Group	Tr1 - Open	Underpass 1	Underpass 2	Underpass 3
WT M	14 ± 1 s	18 ± 6 s	26 ± 4 s	41 ± 4 s
WT F	19 ± 5 s	38 ± 16 s	19 ± 6 s	41 ± 14 s
HET M	26 ± 7 s	16 ± 4 s	29 ± 6 s	30 ± 6 s
HET F	17±5 s	9 ± 2 s	14 ± 3 s	23 ± 9 s
KO M	24 ± 3 s	16 ± 3 s	12 ± 5 s	15 ± 4 s
KO F	20 ± 3 s	15 ± 4 s	9 ± 1 s	19 ± 8 s

Table 22: Mean latency (s) to "goal" box on day 2 of puzzle box

Group	Tr1 – Sawdust1	Sawdust1 1	Sawdust1 2	Sawdust1 3
WT M	10 ± 3 s	42 ± 15 s	76 ± 21 s	126 ± 23 s
WT F	14 ± 3 s	62 ± 18 s	71 ± 16 s	96 ± 12 s
HET M	23 ± 11 s	48 ± 11 s	78 ± 16 s	19 ± 14 s
HET F	17 ± 7 s	45 ± 7 s	36 ± 11 s	46 ± 11 s
KO M	10 ± 1 s	51 ± 13 s	21 ± 7 s	28 ± 7 s
KO F	8 ± 2 s	40 ± 15 s	34 ± 12 s	23 ± 9 s

Table 23: Mean latency to "goal" box on day 3 of puzzle box

Group	Tr1 – Sawdust1	Sawdust2 1	Sawdust2 2	Sawdust2 3
WT M	131 ± 20 s	97 ± 27 s	100 ± 5 s	108 ± 16 s
WT F	74 ± 25 s	100 ± 18 s	120 ± 20 s	142 ± 18 s
HET M	120 ± 19 s	113 ± 16 s	108 ± 14 s	103 ± 15 s
HET F	59 ± 21 s	66 ± 18 s	91 ± 16 s	96 ± 13 s
KO M	26 ± 9 s	44 ± 13 s	42 ± 11 s	62 ± 18 s
KO F	16 ± 5 s	30 ± 12 s	29 ± 9 s	56 ± 11 s

Table 24: Mean latency to "goal" box on day 4 & 5 of puzzle box

Group	Tr1 – Sawdust2	Plug 1	Plug 2	Plug 3	Plug 4	Day 5 - Plug
WT M	136 ± 28 s	118 ± 20 s	63 ± 9 s	94 ± 17 s	119 ± 19 s	172 ± 7 s
WT F	147 ± 19 s	141 ± 20 s	137 ± 15 s	160 ± 10 s	177 ± 3 s	174 ± 6 s
HET M	161 ± 9 s	126 ± 15 s	79 ± 13 s	110 ± 14 s	128 ± 16 s	149 ± 12 s
HET F	115 ± 18 s	135 ± 13 s	104 ± 17 s	103 ± 15 s	133 ± 15 s	156 ± 12 s
KO M	98 ± 22 s	83 ± 21 s	39 ± 8 s	43 ± 16 s	41 ± 10 s	121 ± 19 s
KO F	65 ± 23 s	176 ± 3 s	98 ± 22 s	69 ± 7 s	73 ± 18 s	134 ± 11 s

Appendix 2

Additional means ± sem from behavioural tasks in chapter 4.

6.5.6 Olfactory habituation-dishabituation task

Table 25: Mean ± sem duration of sniffing across trials in the olfactory habituation-dishabituation task in sham and trim rats.

Trials consist of: water trial 1 (W1), water trial 2 (W2), water trial 3 (WR), banana trial 1 (B1), banana trial 2 (B2), banana trial 3 (B3), almond trial 1 (A1), almond trial 2 (A2), almond trial 3 (A3), social odour trial 1 (S1), social odour trial 2 (S2), social odour trial 3 (S3), new social odour trial 1 (NS1), new social odour trial 2 (NS2), new social odour trial 3 (NS3). Duration of sniffing is measured in seconds (s).

Group	W1	W2	W3	B1	B2	B3	A1	A2	A3	S1	S2	S3	NS1	NS2	NS3
Sham	15±4	8±2	15±3	16±3	6±2	7±3	15±4	6±1	6±2	10±2	8±2	7±3	10±2	9±5	3±1
Trim	14±3	7±2	6±2	12±2	7±3	8±2	14±2	8±3	10±4	17±4	13±4	6±2	10±4	10±4	4±2